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14. ABSTRACT
The purpose of this study was to assess the potential for brief periods of low magnitude high frequency mechanical stimulation signals in the musculoskeletal system. The major findings were that short bouts of extremely low-level mechanical signals, several orders of magnitude below that associated with vigorous exercise, increased bone and muscle mass in the weight bearing skeleton of young adult females with low bone density. Ultimately, this information could be of great benefit to enhance musculoskeletal development and decrease the risk for stress fractures in military recruits. Moreover, should these musculoskeletal persist through adulthood, this intervention may prove a deterrent to osteoporosis in the elderly.

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mechanical intervention, fractures, IGF-I, teenagers, low bone mass

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Table of Contents

INTRODUCTION

Susceptibility for low bone mass is present early in life, the amount of bone gained during adolescence is a main contributor to peak bone mass in the young adult, and peak bone mass in the young adult is a likely determinant of osteoporosis in the elderly. While research continues to identify means of reversing osteoporosis in the elderly, these data from children, adolescents and young adults indicate that enhancing bone health early in life represents a viable means of deterring osteoporosis decades before it arises. However, the benefits of early pharmacological interventions to prevent a disease that will not manifest for decades must be weighed against the possible complications of extended treatment.

Approximately one in three children suffer a bone fracture by the time they reach skeletal maturity. While strenuous physical activity and occupational hazards are key factors in the pathogenesis of these fractures, several studies indicate that teenagers who sustain fractures also have decreased bone mass. Therefore, the use of low-level mechanical signals to strengthen bone in young subjects with low bone mass may be relevant not only to the treatment of existing skeletal fragility, but, by enhancing peak bone mass – and retaining it through adulthood – may reduce the risk of osteoporosis and fractures later in life. This study was designed to establish if brief, daily exposure to extremely low-level mechanical stimuli is anabolic to musculoskeletal development in young males and females, 15-20 years of age, with low bone density, who had previously sustained a fracture.

The effects of two twelve-month interventions on musculoskeletal development in young men and women are being longitudinally studied and the results compared to matched groups of subjects undergoing no intervention. The mechanical intervention consists of brief exposure to low level (0.3g; 1g = earth gravitational field) high frequency (30-Hz) mechanical loading for 10 minutes every day. The resistance exercise intervention consists of 30 minutes of weight-bearing and trunk stabilization exercises three times per week.

The cross-sectional properties of the bone make a substantial contribution to its strength. Data indicate that the cross-sectional dimensions of bone are important determinants of low-energy impact fractures in children, stress fractures in military recruits, and osteoporotic fractures in elderly women. Insulin-like growth factor-I (IGF-I), a major regulator of longitudinal bone growth, has also recently been shown to be an important determinant of cross-sectional bone growth. This study also examines the possible relations between the cross-sectional properties of bone and circulating levels of IGF-I, IGF-binding protein-3, and IGF-I genotypes in young adults who had previously sustained fractures. The possible relations between bone acquisition induced by mechanical stimulus and circulating levels of IGF-I and the IGF-I genotype are being assessed.

BODY

Cross-sectional Study – *Females & Males*.

As previously reported, the cross-sectional phase of this study was completed in 2004; 144 females and 144 males participated. Subjects underwent physical examinations to confirm completion of sexual development, anthropometric measurements, x-rays of the left hand/wrist for skeletal age, blood draws for IGF-I, IGFBP-3, IGF-I genotyping, measurements of bone and body composition obtained via computed tomography (CT) and dual energy x-ray absorptiometry (DXA), and questionnaires pertaining to dietary intake and physical activity. Results and conclusions from the cross-sectional studies were included in the October 2004 Annual Report.

Longitudinal Study – *Females*.

Of the 144 women who volunteered for this project, those with the lowest values for bone were enrolled in the longitudinal arm, which entailed three groups: control, vibration intervention, and physical activity intervention. Table 1 illustrates the baseline characteristics of the subjects in each group.

Table 1. Baseline measures for
anthropometric parameters, physical activity, and calcium intake
for the female controls, vibration, and physical activity intervention groups.

	Control	Vibration Intervention	Activity Intervention
Age (yrs)	17.6 ± 1.3	17.3 ± 1.5	19.6 ± 1.6
Bone Age (yrs)	17.4 ± 0.7	17.0 ± 1.0	18.0 ± 0.1
Height (cm)	164.0 ± 6.1	160.8 ± 3.8	161.3 ± 6.3
Weight (cm)	67.5 ± 15	63.3 ± 13.7	63.4 ± 20.0
BMI (kg/m^3)	25.1 ± 5.5	24.5 ± 5.5	24.2 ± 6.3
Physical Exercise Index (hr/week)	9.9 ± 9.0	11.3 ± 11	14.7 ± 10.5
Inactivity Index (hr/week)	8.9 ± 9.3	5.6 ± 3.9	8.1 ± 4.3
Calcium Intake (mg/day)	1138 ± 814	1354 ± 1251	1201 ± 724

Mechanical Stimulation and Control Arms. Results of studies in the control and vibration intervention groups are detailed below.

Intention to Treat Analysis: Over the course of the one year intervention, experimental and control subjects showed identical increases in height (0.4%), and similar increases in weight (2.6% and 2.1%, respectively), BMI (1.9% and 1.4%, respectively) and calcium intake (42% and 36%, respectively), with no significant differences at follow-up in measures of physical activity or inactivity. There were no reported adverse reactions to the mechanical intervention treatment.

Table 2 summarizes the results from the ITT analysis, with baseline and follow-up CT values for muscle and bone in the axial and appendicular skeleton for both groups. Baseline values for the panel of musculoskeletal measures were not significantly different in the experimental group than those measured in the controls. While significant increases were present at follow-up for all

morphological traits in experimental group, the only significant change observed in the control group was evident in the cross-sectional area of the femur.

Table 2. Baseline, short-term (1-year) and long-term (2-year) CT measures and P values for specific musculoskeletal regions within the axial and appendicular skeleton of both control and experimental groups ($N = 24$ in each group).

	Control			Experimental		
Axial	**Baseline**	**One Year**	**Two Years**	**Baseline**	**One Year**	**Two Years**
Total Paraspinous Musculature (cm^2)	181.6 ± 26	182.8 ± 27	184.5 ± 26	167.5 ± 29	177.5 ± 31	178.0 ± 32
Psoas (cm^2)	48.7 ± 8.2	48.7 ± 7.7	49.1 ± 7.9	45.0 ± 9.5	48.0 ± 10.9	48.3 ± 10.5
Quadratus Lumborum (cm^2)	20.9 ± 5.9	21.9 ± 6.7	21.8 ± 6.5	19.1 ± 3.6	21.2 ± 4.3	21.7 ± 4.5
Erector Spinae (cm^2)	112.0 ± 15.0	112.2 ± 15.0	112.1 ± 15.0	103.4 ± 21	108.3 ± 21	108.7 ± 22
Spine Cancellous Bone Density (mg/cm^3)	171.3 ± 17.1	171.5 ± 14.9	171.6 ± 13.8	164.8 ± 25	168.6 ± 25	168.8 ± 25
Appendicular						
Quadriceps femoris muscle (cm^2)	112.0 ± 16.0	114.6 ± 14.0	114.8 ± 17.0	104.4 ± 13	108.5 ± 15	109.2 ± 14
Femur Cross-sectional Area (cm^2)	5.12 ± 0.77	5.17 ± 0.82	5.18 ± 0.79	4.82 ± 0.53	4.92 ± 0.52	4.94 ± 0.53
Femur Cortical Bone Area (cm^2)	4.18 ± 0.51	4.24 ± 0.58	4.30 ± 0.50	3.96 ± 0.43	4.10 ± 0.42	4.14 ± 0.41

Table 3 presents the absolute changes and percent changes for all women in each of the two groups. In the axial skeleton, significantly greater increases were evident in the absolute and/or percent change of paraspinous musculature of the experimental group over all controls, with 6.0% greater gains measured in the psoas ($p<0.003$) and 4.4% in the erector spinae ($p=0.03$). The spine had 2.0% more cancellous bone in the experimental than the control cohort ($p=0.06$).

Table 3. After the 1-year intervention, absolute and percent change in CT measures of specific musculoskeletal regions of the axial and appendicular skeleton for all subjects in the control and experimental groups ($N = 24$ in each group).

	Absolute change			*Percent change*		
Axial	**Control**	**Experimental**	**P**	**Control**	**Experimental**	**P**
Total Paraspinous Musculature (cm^2)	1.2 ± 9.0	10.1 ± 12.5	0.007	0.5 ± 5.0	5.4 ± 6.9	0.002
Psoas (cm^2)	0.0 ± 2.9	3.1 ± 3.5	0.002	-0.1 ± 0.1	5.9 ± 6.7	0.003
Quadratus Lumborum (cm^2)	1.0 ± 2.7	2.2 ± 2.6	0.16	3.0 ± 14.7	9.0 ± 11.7	0.17
Erector Spinae (cm^2)	0.2 ± 5.6	5.3 ± 11.0	0.05	-0.1 ± 0.9	4.3 ± 8.8	0.03
Spine Cancellous Bone Density (mg/cm^3)	0.1 ± 7.7	3.8 ± 7.7	0.11	0.1 ± 4.5	2.1 ± 4.9	0.06
Appendicular						
Quadriceps Femoris Area (cm^2)	2.6 ± 8.4	4.1 ± 4.5	0.45	2.2 ± 2.7	3.6 ± 3.6	0.36
Femur Cross-sectional Area (cm^2)	0.1 ± 0.1	0.1 ± 0.2	0.25	0.9 ± 2.2	1.9 ± 3.4	0.28
Femur Cortical Bone Area (cm^2)	0.05 ± 0.17	0.14 ± 0.15	0.08	1.1 ± 3.7	3.4 ± 3.7	0.04

In the appendicular skeleton, experimental subjects had a 2.3% greater increase than controls in femoral cortical bone area ($p<0.04$; Figure 1). Considering that the cross sectional area defined by the periosteal envelope (femur cross-sectional area) was similar in the two groups (mean area increase in each cohort increased 0.1 cm^2; $p=0.25$), indicates that the increase in bone area was achieved through apposition on the endosteal surface.

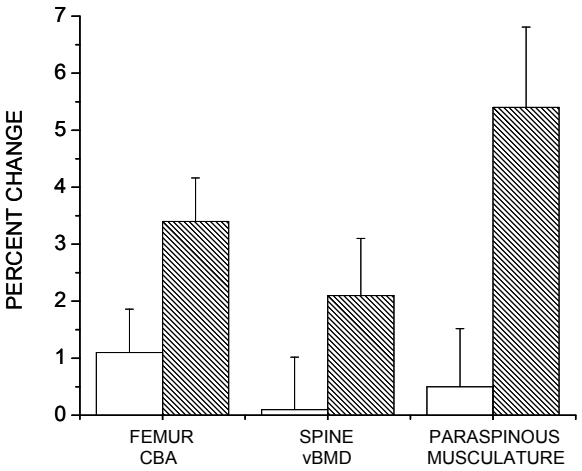

Figure 1. Percent change (mean ± SE) occurring over the 1-year intervention from both the control (white bars) and experimental (striped bars) subjects, using an intention-to-treat analysis and therefore including all 24 subjects who began the protocol in each group. The graph presents the CT data from the cortical bone area of the femur ($p = 0.04$), the cancellous BMD of the spine ($p = 0.06$), and the total paraspinous musculature ($p = 0.002$).

None of the baseline variables showed a significant correlation with any of the absolute or percent changes over the 12-month experimental period. As a result, p-values changed insignificantly when any of these baseline characteristics were considered as covariates for the absolute and relative comparison between controls and experimental subjects.

Statistically significant differences between experimental subjects and controls were also found when the changes from all outcome variables were analyzed as a vector of observation using a multivariate repeated measure ANOVA; this was true whether the analysis was based on absolute change or percent changes, with or without covariates ($p<0.05$). When separated into two anatomical regions, significant differences were observed for the axial, but not for the appendicular skeleton.

Per Protocol Analysis: Compliance in the 24 women in the experimental group was highly variable, ranging from 1-100%, with a mean compliance of 130.3 ± 92.1 min/month or 4.3 min/day (Figure 2a). A post-hoc, per-protocol analysis was used to determine if a there was a dose:response benefit of treatment duration, or if a compliance threshold existed, beyond which exposure to mechanical intervention no longer provided additional benefit. The experimental cohort was stratified into quartiles according to their percent compliance, with the bottom quartile including compliance values between 1-13% (n=6), the second lowest quartile of compliance between 21-39% (n=6), the second highest quartile fell between 41-71% of compliance (n=6), and the quartile with the highest compliance was between 77-100% of compliance (n=6).

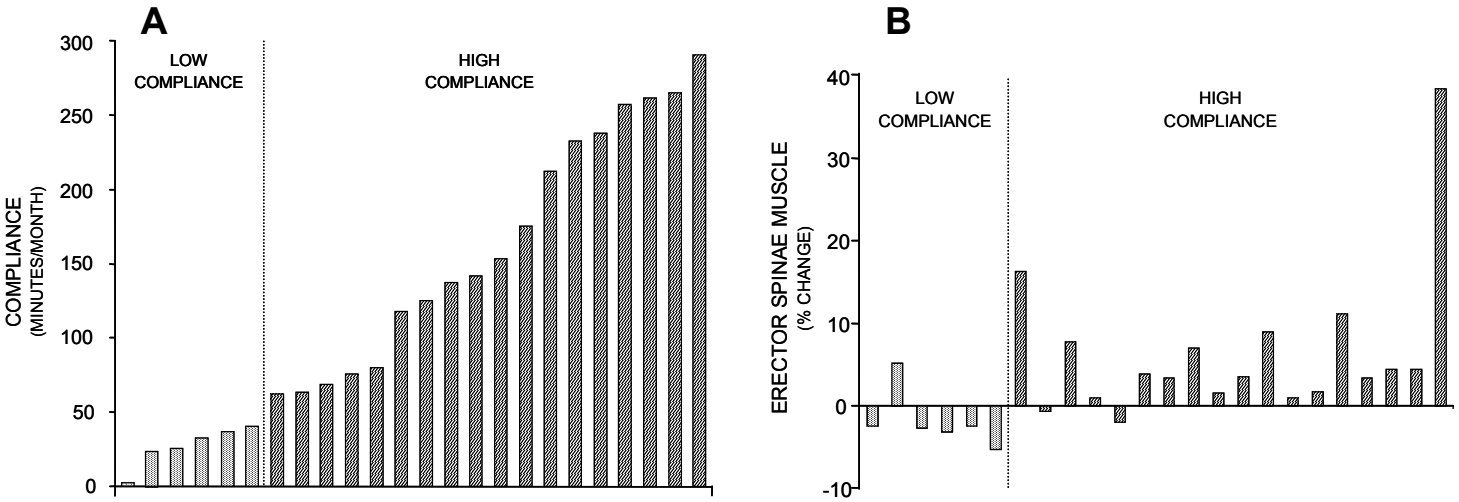

Figure 2. A) Compliance for each of the 24 subjects in the experimental group, as expressed in minutes per month. Each subject was requested to use the device for 10 minutes/day, such that 300 minutes/month would represent 100% compliance. Experimental subjects are represented either as those who used the device <20% of the allotted time (stippled bars) and are indicated as low compliance ($N = 6$) or those who used the device for >20% of the time (striped bars) and are indicated as high compliance ($N = 18$). B) Percent change in the cross-sectional area of the erector spinae muscle of each experimental subject, as related to their compliance.

A dose effect was evident in the erector spinae muscle, providing a first indication of a significant increase in muscle mass achieved at 20% compliance (two minutes per day; Figure 2b). When assessed via the responsivity of specific quartiles of compliance, clear threshold characteristics were observed in a number of musculoskeletal sites, with the lowest quartile

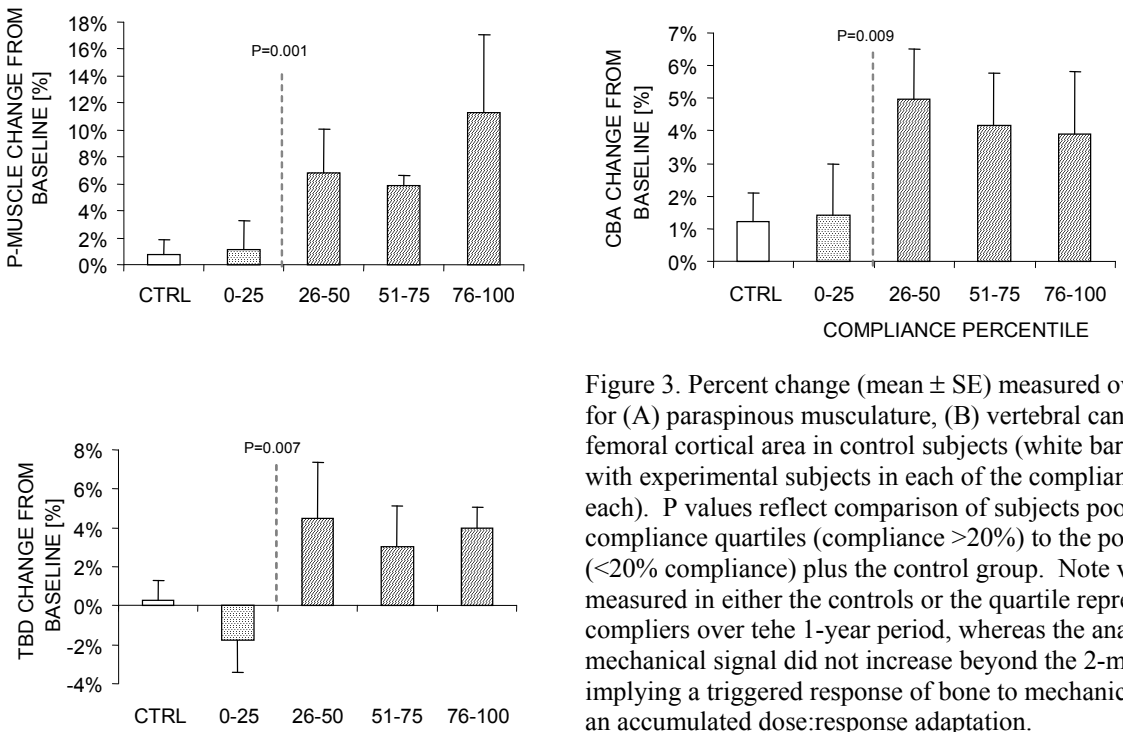

Figure 3. Percent change (mean ± SE) measured over the 1-year period for (A) paraspinous musculature, (B) vertebral cancellous BMD, and (C) femoral cortical area in control subjects (white bars; N = 24) compared with experimental subjects in each of the compliance quartiles (N = 6 each). P values reflect comparison of subjects pooled from the three top compliance quartiles (compliance >20%) to the pooled low compliance (<20% compliance) plus the control group. Note very little change was measured in either the controls or the quartile representing the lowest compliers over tehe 1-year period, whereas the anabolic response to the mechanical signal did not increase beyond the 2-minute "threshold", implying a triggered response of bone to mechanical signals rather than an accumulated dose:response adaptation.

failing to respond at all to the intervention, and the three highest quartiles being very similar in their responses (Figure 3). Given the non-responsivity of those in the lowest quartile of compliance, these subjects were pooled with controls. Moving these low compliers into the control groups further reduced the small differences in baseline characteristics between control and experimental subjects, including the p-value for the difference in height from less than 0.05 to p=0.8.

There were no significant changes between short-term and long-term visits in any of the parameters measured in the axial or appendicular skeletons. This was true for bone, muscle and fat measures, regardless of whether assessments were made as percent change or absolute change.

As summarized in Table 4, women who used the intervention at least two minutes per day (n=18) showed significant increases over the group pooling controls and those in the lowest quartile of compliance (n=30). Figure 4 illustrates the differences between groups, and includes an 8.3% greater cross sectional area of the erector spinae musculature in highly compliant women over controls and low compliers (p=0.006), a 5.2% increase in the cross sectional area of the psoas (p=0.02), 7.2% greater mass in the total paraspinous musculature of high compliers (p=0.001), a 3.9% greater density in the cancellous bone of the spine (p=0.007), and a 2.9% greater cortical bone area in the femur (p=0.009). No significant differences were observed in the musculature of the femur, or in the cross-sectional area - in contrast to cortical bone area - of the femur.

Table 4. Using a Per Protocol analysis, subjects (n=6) within the lowest quartile of compliance were pooled with controls (controls + poor compliers: total n=30), and compared to the absolute and percent changes measured from CT in the subjects in the three highest quartiles of compliance (high compliers: n=18). Highly significant differences were observed in several regions of the spine musculature, as well as the cancellous bone of the spine and cortical bone area of the hip, while musculature around the femur and cross-sectional area of the femur were not significantly different between groups.

	Absolute Change			*Percent Change*		
Axial	**Control + Poor-Comp.**	**High-Compliers**	**P**	**Control + Poor-Comp.**	**High-Compliers**	**P**
Total Paraspinous Musculature (cm^2)	1.4 ± 8.9	12.6 ± 12.6	0.001	0.8 ± 5.1	8.0 ± 9.1	0.001
Psoas (cm^2)	0.6 ± 3.6	3.1 ± 2.8	0.01	1.6 ± 8.2	6.8 ± 6.0	0.02
Quadratus Lumborum (cm^2)	1.1 ± 2.5	2.4 ± 2.7	0.11	5.4 ± 13.7	13.4 ± 15.0	0.07
Erector Spinae (cm^2)	-0.3 ± 5.3	7.1 ± 10.4	0.002	-0.2 ± 4.7	8.1 ± 14.5	0.006
Spine Cancellous Bone Density (mg/cm^3)	-0.4 ± 7.4	5.9 ± 7.2	0.006	-0.1 ± 4.5	3.8 ± 4.9	0.007
Appendicular						
Quadriceps Femoris Area (cm^2)	3.0 ± 7.8	4.0 ± 4.5	0.59	3.0 ± 6.8	3.9 ± 4.2	0.63
Femur Cross-sectional Area (cm^2)	0.05 ± 0.12	0.12 ± 0.16	0.10	1.0 ± 2.2	2.4 ± 3.7	0.12
Femur Cortical Bone Area (cm^2)	0.05 ± 0.17	0.17 ± 0.13	0.02	1.3 ± 3.9	4.3 ± 3.6	0.009

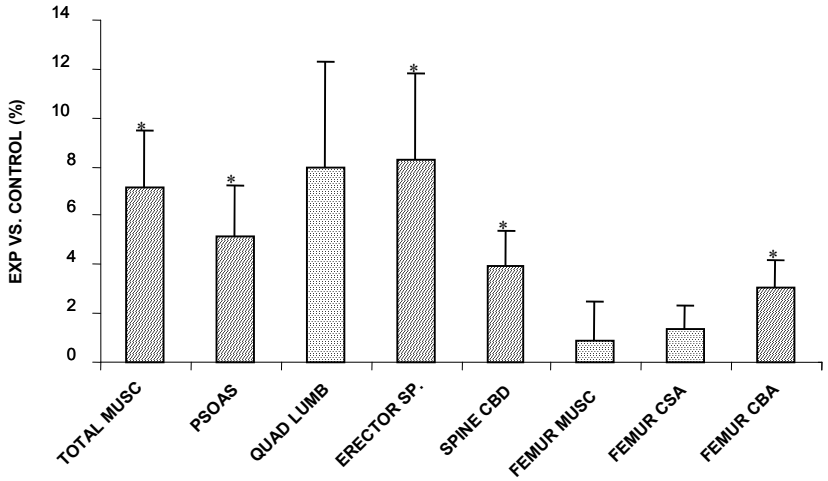

Figure 4. Differences in the change (mean + SE) measured over the 1-year period for those who used the device >2 minutes/day, as compared with the controls pooled with the women in the lowest quartile of compliance. Each parameter evaluated, with the exception of musculature around the femur and femoral cross-sectional area, showed that the experimental group benefited significantly (*) from the mechanical intervention.

DXA: Mean values for spine bone mineral content (BMC) and area bone mineral density (aBMD) and for total body BMC were significantly higher in both groups at follow-up. In addition, in the experimental group, values for total body aBMD were higher after the intervention. There were, however, no significant differences between groups in the absolute and/or percent change for any of these DXA measures of bone and body composition.

Longitudinal Study – *Males.*

Of the 144 males who volunteered to participate in this project, those with the lowest values for bone were enrolled in one of three groups in the longitudinal arm: control, vibration intervention, or physical activity intervention. Table 5 illustrates the baseline characteristics of the subjects in each group.

Table 5. Baseline measures for anthropometric parameters, physical activity, and calcium intake for the male controls, vibration, and physical activity intervention groups.

	Control	Vibration Intervention	Activity Intervention
Age (years)	17.4 ± 1.4	17.0 ± 1.9	20.3 ± 1.7
Skeletal Age (years)	17.3 ± 0.9	17.0 ± 1.1	18.9 ± 0.1
Height (cm)	173.0 ± 8.7	170.0 ± 8.5	175.0 ± 7.5
Weight (kg)	75.0 ± 14.3	64.6 ± 16.8	77.2 ± 12.6
BMI (kg/m2)	25.1 ± 5.0	22.2 ± 4.2	25.2 ± 3.8
Physical Exercise Index (hr/wk)	11.7 ± 9.4	13.2 ± 12.1	13.6 ± 7.8
Inactivity Index (hr/wk)	6.9 ± 3.7	11.1 ± 16.1	9.00 ± 4.2
Calcium Intake* (mg/day)	950.6 ± 595	1063.7 ± 443	1001.0 ± 607

Mechanical Stimulation and Control Arms. Twenty-four males were initially enrolled in each of the vibration intervention and control groups. During the course of the intervention, one participant in the vibration intervention group moved out of state and one was incarcerated; by February 2006, the remaining 22 males had completed the intervention.

Table 6 shows baseline and follow-up values for measures of bone, muscle and fat in the axial and appendicular skeleton for the control and mechanical intervention groups, and Table 7 describes the absolute and percent change for CT values in the control and experimental groups.

Table 6. Baseline and 1-year CT measures and P values for specific musculoskeletal regions within the axial and appendicular skeleton for both control (*N*=24) and experimental (*N*=21) groups

	Control			Experimental		
	Baseline	**Follow-up**	**P**	**Baseline**	**Follow-up**	**P**
Axial						
Paraspinous Musculature (L2+L3)	91.7 ± 14.8	92.3 ± 15.3	0.63	80.5 ± 20.1	84.3 ± 18.7	0.01
Spine Cancellous BMD (mg/cm^2)	171.9 ± 26.9	176.5 ± 29.2	0.06	158.1 ± 26.7	165.4 ± 32.7	0.08
Spine cross-sectional area (cm^2)	10.7 ± 1.4	10.8 ± 1.4	0.24	10.6 ± 1.4	10.6 ± 1.6	0.92
V-Fat (cm^2)	43.1 ± 62.0	57.4 ± 94.4	0.05	15.7 ± 20.5	23.6 ± 39.0	0.09
S-Fat (cm^2)	174.0 ± 149.6	194.7 ± 173.7	0.04	104.9 ± 120.6	116.3 ± 116.4	0.40
Total Fat (cm^2)	217.1 ± 207.8	252.1 ± 261.2	0.02	120.2 ± 138.0	140.0 ± 153.3	0.22
Vertebral Height (cm)	2.5 ± 0.2	2.5 ± 0.2	0.12	2.4 ± 0.1	2.5 ± 0.1	0.003
Vertebral Volume (mg/cm)	26.5 ± 5.1	27.3 ± 4.9	0.04	26.1 ± 4.4	26.8 ± 5.3	0.06
Appendicular						
Quadriceps femoris area (cm^2)	138.4 ± 16.0	158.7 ± 18.9	<.001	129.4 ± 25.3	147.0 ± 36.3	<.001
Femur cross-sectional area (cm^2)	6.4 ± 0.6	6.5 ± 0.7	.002	5.8 ± 0.8	6.0 ± 0.9	.002
Femur cortical bone area (cm^2)	5.1 ± 0.6	5.3 ± 0.6	<.001	4.7 ± 0.7	4.9 ± 0.8	<.001
Femur BMD (mg/cm^2)	1194.8 ± 33.2	1204.9 ± 44.5	0.35	1193.4 ± 27.7	1208.7 ± 41.5	0.08
Femur Fat (cm2)	61.5 ± 35.8	64.2 ± 36.5	0.62	41.1 ± 28.0	53.5 ± 33.2	0.09

Table 7. Absolute and percent change for CT measures and P values for specific musculoskeletal regions within the axial and appendicular skeleton for both control (N=24) and experimental (N=21) groups.

	Absolute Change			*Percent Change*		
	Control	**Experimental**	**P**	**Control**	**Experimental**	**P**
Axial						
Paraspinous Musculature (L2+L3)	0.6 ± 7.2	3.8 ± 6.3	0.12	0.3 ± 8.3	4.7 ± 8.3	0.08
Spine Cancellous BMD (mg/cm^2)	4.7 ± 11.7	6.9 ± 17.0	0.60	2.3 ± 7.9	3.1 ± 9.2	0.74
Spine cross-sectional area (cm^2)	0.1 ± 0.4	0.01 ± 0.4	0.37	0.7 ± 3.4	0.1 ± 3.1	0.30
V-Fat (cm^2)	14.3 ± 34.7	7.9 ± 20.2	0.46	16.2 ± 36.8	9.0 ± 73.0	0.67
S-Fat (cm^2)	20.7 ± 46.9	11.4 ± 61.0	0.57	3.2 ± 29.3	7.5 ± 47.6	0.72
Total Fat (cm^2)	35.0 ± 71.4	19.7 ± 71.0	0.48	6.6 ± 28.1	9.1 ± 47.0	0.83
Vertebral Height (cm)	0.04 ± 0.1	0.07 ± 0.17	0.39	1.7 ± 3.9	2.7 ± 4.0	0.39
Vertebral Volume (mg/cm)	0.8 ± 1.7	0.8 ± 1.8	0.99	2.9 ± 6.2	2.4 ± 6.0	0.78
Appendicular						
Quadriceps femoris area (cm^2)	20.3 ± 10.6	17.6 ± 17.3	0.53	12.5 ± 6.2	11.0 ± 8.2	0.49
Femur cross-sectional area (cm^2)	0.2 ± 0.2	0.1 ± 0.2	0.53	2.6 ± 3.3	2.2 ± 2.9	0.70
Femur cortical bone area (cm^2)	0.1 ± 0.1	0.2 ± 0.2	0.26	2.9 ± 3.0	4.3 ± 4.0	0.18
Femur BMD (mg/cm^2)	10.2 ± 52.1	15.3 ± 37.7	0.71	0.7 ± 4.4	1.2 ± 3.1	0.69
Femur Fat (cm2)	6.9 ± 16.7	12.3 ± 31.7	0.47	9.9 ± 24.5	14.0 ± 44.3	0.70

There were no significant differences in any of the measures.

Figure 5 depicts compliance in minutes per month for all males in the mechanical stimulation intervention group. We found that males were less compliant than females had been.

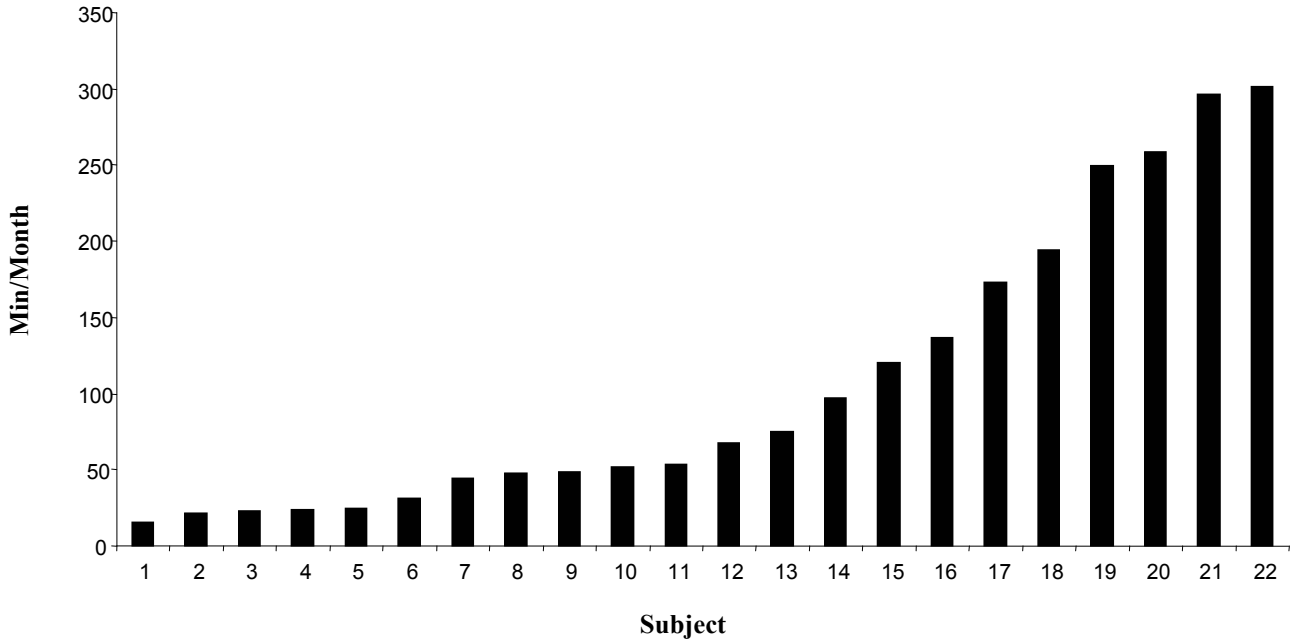

Figure 5. Compliance of the male subjects completing this study expressed as minutes/month.

Physical Exercise Arm - Females & Males. As previously reported, the physical activity arm was severely delayed. Following IRB approval in late 2005, female and male participants selected a gym near their homes and arrangements were made for their membership. Thereafter, subjects began 30 minutes of weight-bearing exercise three times/week for this one-year intervention. All subjects were provided with TUMS 500 mg for daily intake during their participation. Weekly telephone calls take place to encourage and record compliance. It is anticipated that all participants in this group will complete the intervention by June 2007.

IGF-I Levels and Measures of Bone Structure. Serum levels of IGF-I were examined prior to and following the mechanical intervention in both study subjects and controls. At the mid-shaft of the femurs, IGF-I did not correlate with the material density of cortical bone ($r = -0.08$), but did correlate significantly with cortical bone area ($r = 0.50$; $P < 0.0001$) and with the cross-sectional area ($r = 0.49$; $P < 0.0001$) of the bone. When using multiple regression analyses, IGF-I was associated with both the cross-sectional area ($P = 0.03$) and cortical bone area ($P= 0.04$), even after accounting for age, gender, weight and the length of the femur. Thus, in the appendicular skeleton of male and female teenagers and young adults in this study, IGF-I had no influence on the material density of the bone, but was found to be a major determinant of the cross-sectional properties of the bone.

Positive Findings
Low intensity, high frequency mechanical vibration enhances bone and muscle mass in young women. In contrast, no significant differences were found in bone acquisition between young men undergoing high frequency mechanical stimulation and controls.

Negative Findings
The intervention was not associated to any adverse side effects. There were no associations observed between calcium intake and measures of physical exercise and bone and muscle measures in the control or intervention groups.

KEY RESEARCH ACCOMPLISHMENTS

- Baseline studies in 144 females and 144 males completed.
- Mechanical intervention arm of the longitudinal study and short-term and long-term post-intervention examinations are completed in females.
- Control arm of the longitudinal study and short-term and long-term post-intervention examinations in females completed.
- Mechanical intervention arm of the longitudinal study and short-term post-intervention examinations are completed in males.
- Control arm of the longitudinal study and short-term examinations in males are completed.
- The exercise intervention arm in females and in males is in process.

REPORTABLE OUTCOMES

Publications, Abstracts and Presentations

Wren, TA, Liu X, Pitukcheewanont P, Gilsanz V. Bone densitometry in pediatric populations: discrepancies in the diagnosis of osteoporosis by DXA and CT. J Pediatr. 146:776-779, 2005.

Gilsanz V, Wren TAL, Sanchez M, Dorey F, Judex S, Rubin C. Low level, high frequency mechanical signals enhance musculoskeletal development of young women with low BMD. J Bone Miner Res. 21:1464-1474, 2006.

Janicka A, Wren TAL, Mittelman S, Sanchez M, Dorey F, Gilsanz V. Fat mass is not beneficial to bone in adolescents and young adults. J Clin Endocrinol Metab, in press, 2006.

10/2004 "Low DXA and CT Bone Measures in Young Adults with a Simple Sequence Repeat in IGF-I Gene"
26th Annual Meeting of the American Society for Bone and Mineral Research
Seattle, WA

03/2005 "Comparison of CT and DXA Measurements in Healthy Children"
Bone Mineral Density in Childhood Study (BMDCS) Meeting
National Institute of Child Health and Human Development
Bethesda, MD

09/2005 "Mechanical Intervention Enhances Bone and Muscle in Young Women with Low Bone Density"
American Society of Bone and Mineral Research 27th Annual Meeting

06/2006 "Mechanical Intervention Enhances Bone and Muscle in Young Women with Low Bone Density"
CHLA Saban Research Institute 11th Annual Poster Session

06/2006 "Fat Mass is Not Beneficial to Bone"
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06/2006 "Assessment of Vertebral Peak Bone Mass by CT and DXA"
CHLA Saban Research Institute 11th Annual Poster Session

09/2006 "Good, Good, Good Vibrations: Evidence for the Therapeutic Potential of Low-Magnitude, High Frequency Mechanical Signals"
American Society of Bone and Mineral Research 28th Annual Meeting

CONCLUSIONS

The results in female subjects indicate that mechanical signals at orders of magnitude below that which might cause damage to bone tissue can have a strong anabolic effect on musculoskeletal development. On average, CT measures of cancellous bone in the axial skeleton and of cortical bone in the appendicular skeleton increased 2.1% and 2.3% more, respectively, in subjects treated with low-magnitude mechanical loading than in controls. Simultaneous to gains in bone, low-magnitude high-frequency vibration significantly increased muscle mass; close to a 5% greater increase in CT values for paraspinous musculature was detected in women in the intervention group, compared to controls. An association was observed between musculoskeletal gains and compliance; women using the vibration system more than 2 min/day had greater gains in cancellous and cortical bone and paraspinous musculature than women using it less than 2 min/day, or not at all.

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BONE DENSITOMETRY IN PEDIATRIC POPULATIONS: DISCREPANCIES IN THE DIAGNOSIS OF OSTEOPOROSIS BY DXA AND CT

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Objectives To test the hypothesis that because of errors associated with growth and development, osteoporosis is frequently overdiagnosed in children when using dual-energy x-ray absorptiometry (DXA). This study compared bone density values obtained by DXA with those from computed tomography (CT), which is not influenced by body or skeletal size.

Study design Vertebral bone density was measured by using both DXA and CT in 400 children (100 each, healthy and sick boys and girls). Regression analysis was used to compare DXA and CT *Z* scores, and the agreement between DXA and CT classifications of *Z* scores below −2.0 was examined.

Results DXA and CT *Z* scores were moderately related (r^2 = 0.55 after accounting for age and anthropometric measures). DXA *Z* scores predicted CT *Z* scores below −2.0 with reasonable sensitivity (72%), specificity (85%), and negative predictive value (98%), but positive predictive value was low (24%). Many more subjects were classified as having bone density lower by DXA (76/400) than by CT (25/400), particularly subjects below the 5th percentile of height and/or weight for age.

Conclusions The inability of DXA to account for the large variability in skeletal size and body composition in growing children greatly diminishes the accuracy of this projection technique for assessing bone acquisition and diagnosing osteoporosis in pediatric populations. ***(J Pediatr 2005;146:776-9)***

Dual-energy x-ray absorptiometry (DXA) is the most widely used technique for measuring bone acquisition in children because of its low cost, minimal radiation exposure, accessibility, and ease of use.[1] The availability of DXA has resulted in many large-scale studies of the genetic and environmental determinants of areal bone mineral density (aBMD) in healthy children.[2-13] Although DXA studies in pediatrics have provided much information regarding changes in aBMD over time, there is still considerable confusion over the interpretation of DXA measures. Most growth-related increases in DXA aBMD values are due to increases in the size rather than the density of the bone, and sex differences in aBMD values are also largely the result of greater bone size in male subjects.[14]

The confounding effect of skeletal geometry on DXA measures is gaining much recognition. Recently, it has been suggested that major errors in interpretation occur when using this technique in pediatric populations, leading to the overdiagnosis of osteoporosis in growing subjects. Indeed, several investigators have proposed that osteoporosis should not be diagnosed on the basis of DXA densitometry criteria alone.[15,16] In addition, whereas in adults, DXA aBMD is a powerful predictor of fracture and is used to define osteoporosis, there is insufficient pediatric evidence to determine aBMD standard deviation criteria for osteopenia and osteoporosis, as indicated by the World Health Organization. Hence, it is recommended that when reporting DXA results in subjects younger than 20 years of age, it is more appropriate to define a *Z* score of less than −2.0 as low bone density rather than using the World Health Organization classification for osteoporosis.[15]

In this study, we examined the relation between vertebral DXA measurements of aBMD and vertebral quantitative computed tomography (CT) values of volumetric bone density (vBD), which are not influenced by skeletal or body size, in a large cohort of healthy and sick children. We specifically examined the relation between DXA and CT *Z* scores, which are defined as the number of standard deviations the aBMD or vBD is above or below the mean for age-matched control subjects.

BMD	Bone mineral density	CT	Computed tomography
BMI	Body mass index	DXA	Dual-energy x-ray absorptiometry

See related articles, p 726, p 764, and p 769.

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METHODS

Study Subjects

During the past 5 years, many children and adolescents have had bone measurements through the use of both CT and DXA at Childrens Hospital Los Angeles. This retrospective review included the records from 100 healthy boys and 100 healthy girls who were participants in several studies on bone acquisition during growth and from 100 sick boys and 100 sick girls. For the purpose of this study, "sick" subjects were defined as patients being evaluated for bone mass deficiency. The protocol was approved by the institutional review board for clinical investigations at our institution. Written informed consent was obtained from all healthy subjects and their parents. Data from the sick subjects were reviewed retrospectively under a waiver of consent approved by the institutional review board.

All 400 subjects, ages 6 to 17 years, were enrolled in this study. Age, height, and weight were recorded for each. Measurements of total height were obtained to the nearest 0.1 cm, using the Harpenden stadiometer (Holtain Ltd, Crymmych, Wales), and measurements of weight were obtained to the nearest 0.1 kg, using the Scale-Tronix (Scale-Tronix, Inc, Wheaton, Ill). Height, weight, and body mass index (BMI) percentiles-for-age were determined by using the references provided by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion.[17]

CT and DXA Assessments of Vertebral Bone

The technique for determining lumbar vertebral bone density by quantitative CT has been described in detail elsewhere.[14,18,19] All CT studies were performed by the same radiology technologist, using the same scanner (CT-T 9800; General Electric Co, Milwaukee, WI) and the same mineral reference phantom (CT-T bone densitometry package; General Electric Co, Milwaukee, WI). Identification of the sites to be scanned was performed with lateral scout views, and the density of cancellous bone in the vertebral body was obtained from the 10-mm midportion of the L1, L2, and L3 vertebral bodies. The average density of L1-L3 was calculated and compared with published normative data from our laboratory[20] to determine CT Z score (Z_{CT}). The coefficients of variation for repeated CT measurements of vertebral density are between 0.6% and 1.5%.[21,22] The time required for the procedure was approximately 10 minutes. The radiation dose was approximately 100 to 200 mrem (1.5 mSv), localized to the midportions of the lumbar vertebrae; the effective radiation dose was approximately 8 mrem.[23,24]

Subjects also underwent DXA scanning by the same radiology technologist, using the same densitometer (Delphi W; Hologic, Inc, Waltham, MA). Anterior-posterior scans were obtained for L1-L4. The manufacturer's software calculated aBMD for each vertebral body as well as Z score for the average L1-L4 aBMD (Z_{DXA}). The time required for the procedure was approximately 5 minutes, and the radiation exposure was negligible.[23-25]

Statistical Analysis

Statistical analysis was carried out with the use of Statview (version 5.0.1; SAS Institute Inc, Cary, NC) and Stata (version 8.0; Stata Corp, College Station, TX). Linear regression was used to compare Z_{DXA} with Z_{CT}, both in simple regression and in multiple regression, including age, height, weight, BMI, height percentile, weight percentile, and BMI percentile as covariates. After the regression analysis, the ability of DXA Z scores to predict CT Z scores below -2.0 was examined. Sensitivity (proportion of subjects with CT Z scores below -2.0 who also had DXA Z scores below -2.0), specificity (proportion of subjects with CT Z scores above -2.0 who also had DXA Z scores above -2.0), positive predictive value (proportion of subjects with DXA Z scores below -2.0 who also had CT Z scores below -2.0), and negative predictive values (proportion of subjects with DXA Z scores above -2.0 who also had CT Z scores above -2.0) were calculated.

RESULTS

Table I summarizes the anthropometric measurements for all subjects.

A significant linear relation was observed between Z_{DXA} and Z_{CT} ($r^2 = 0.39$; $P < .0001$) (Figure). This relation was improved when age and anthropometric measures were included in the regression model ($r^2 = 0.55$). Results for subgroups divided by health status (healthy or sick) and sex were similar to the overall results (r^2 values of 0.27 to 0.48 for single regression, 0.51 to 0.65 for multiple regression).

When DXA Z scores were used to predict CT Z scores below -2.0, sensitivity and specificity were reasonable and negative predictive value was extremely high. However, positive predictive value was low (Table II). This was true whether all subjects were analyzed together or sick and healthy subjects were analyzed separately. For the subjects who were classified differently by CT and DXA, many more were identified as having bone density lower by DXA (58/400) than by CT (7/400). Of the 58 subjects who were identified by DXA only, most were small for their age (<5th percentile) in terms of height (30/58, 52%), weight (22/58, 38%), or both height and weight (17/58, 29%).

DISCUSSION

Currently, DXA is routinely used worldwide in children to diagnose osteoporosis, assess response to therapy, and study the determinants of bone accretion during growth. The results of the current study indicate, however, that DXA measures of aBMD underestimate bone accretion in children and adolescents. On average, 3 times as many subjects were determined to have low bone density (Z score <-2.0 for chronological age) by DXA than by CT; this was true for both healthy (2% vs 7%) and sick (10.5% vs 31%) children.

We found that whereas DXA and CT Z scores are related, almost 50% of the variability remains even after age

Table I. Age and anthropometric measures for 400 children

	Healthy		Sick		
	Male (*N* = 100)	Female (*N* = 100)	Male (*N* = 100)	Female (*N* = 100)	All subjects (*N* = 400)
Age (y)	13.7 ± 3.2	13.1 ± 3.1	12.2 ± 3.2	12.0 ± 2.9	12.7 ± 3.2
Height (cm)	160.0 ± 20.1	152.2 ± 15.4	146.8 ± 20.4	141.6 ± 16.8	150.1 ± 19.4
Height percentile	48.3 ± 26.7	50.6 ± 27.6	36.1 ± 33.2	31.8 ± 32.3	41.7 ± 31.0
Weight (kg)	57.3 ± 21.5	49.7 ± 16.0	47.0 ± 21.1	44.6 ± 17.8	49.7 ± 19.8
Weight percentile	62.2 ± 26.9	60.9 ± 24.9	50.9 ± 39.0	51.8 ± 33.9	56.5 ± 32.0
BMI (kg/m^2)	21.5 ± 4.8	20.9 ± 4.5	20.7 ± 5.5	21.5 ± 5.6	21.2 ± 5.1
BMI percentile	63.1 ± 28.3	60.7 ± 26.9	60.6 ± 34.9	66.5 ± 27.7	62.7 ± 29.6

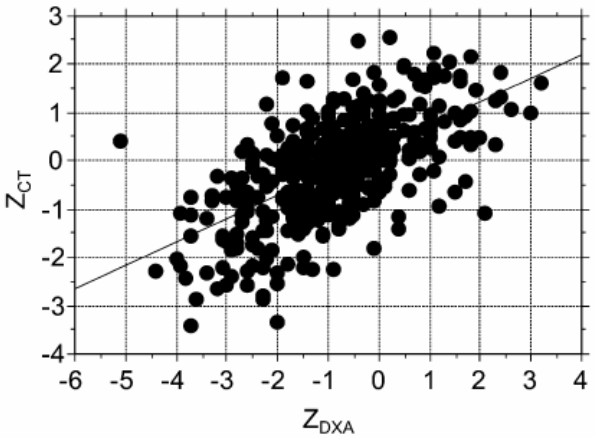

Figure. Linear regression comparing DXA with CT *Z* scores for 400 subjects (r^2 = 0.39).

Table II. Classification of *Z* scores based on cutoff value of −2.0 and classification statistics for prediction of Z_{CT} by Z_{DXA}

Z score <−2	Healthy (*N* = 200)	Sick (*N* = 200)	Total (*N* = 400)
Neither CT/DXA	185	132	317
CT/DXA	3	15	18
CT only	1	6	7
DXA only	11	47	58

	Healthy (*N* = 200)	Sick (*N* = 200)	All subjects (*N* = 400)
Sensitivity	75% (3/4)	71% (15/21)	72% (18/25)
Specificity	94% (185/196)	74% (132/179)	85% (317/375)
Positive PV	21% (3/14)	24% (15/62)	24% (18/76)
Negative PV	99% (185/186)	96% (132/138)	98% (317/324)

PV, predictive value.

and anthropometric measures are taken into account. When classifying low bone density based on a *Z* score cutoff value of −2.0, DXA had a reasonable sensitivity and specificity in predicting CT classification, but positive predictive value was low. This is partly due to DXA underestimating bone density and overestimating osteoporosis in children who are small for their age (<5th percentile for height and/or weight), since bone size tends to increase with greater height and weight. The consequence is that DXA *Z* scores ≥−2 have greater concordance with CT *Z* scores than do DXA *Z* scores <−2, which require further screening to confirm osteoporosis.

Since this study involved two specific bone densitometers, the findings may differ with equipment of other manufactures. The systematic overreading of low bone mass by DXA may, in fact, be the result of the currently available Hologic reference data. When using values from the healthy children in the current study to calculate *Z* scores for the sick children, the tendency of DXA to yield lower *Z* scores than CT was greatly curtailed, although comparisons with this database did not strengthen the correlation between DXA and CT *Z* scores. Although many children were identified as having low bone density by one modality but not the other, they were more evenly split with regard to which technique yielded the <−2 classification. Consequently, discrepancies probably will exist between DXA and CT assessments of low bone density regardless of the reference data used.

In addition, the discrepant results between DXA and CT classifications are, in part, due to the errors associated with the unknown composition of soft tissues adjacent to the axial skeleton. Because corrections for soft tissues are based on the assumption of a homogenous distribution of fat around the vertebrae, changes in DXA measurements are observed if fat is distributed inhomogeneously around the bone measured. It has been estimated that inhomogeneous fat distribution in soft tissues resulting in a difference of 2 cm of fat between the soft tissue and bone areas will influence DXA measurements by 10%.[26] This disadvantage especially limits the use of DXA in studies of children with eating disorders, such as obesity and anorexia nervosa.

Last, the lack of a definable association between pediatric bone density values and a clinical outcome measure obfuscates the significance of these measurements in children. The relation of bone measurements to pediatric fractures is, at

best, debatable, and their association to the risk of osteoporosis and fractures later in life has not yet been defined. However, previous studies have established the constancy of CT percentile measures for bone size and bone density throughout puberty, the time of life in which bone mass more than doubles.[27] Establishing the degree to which BMD values can be tracked throughout childhood and adolescence will help determine whether the identification of children at risk for low peak bone mass is also possible through the use of DXA.

CONCLUSIONS

The interpretation of DXA measurements is considerably more challenging in children and adolescents than in adults because of the dynamic changes in body and skeletal size and configuration associated with growth and sexual development. The results of this study support the contention that current DXA bone determinations frequently underestimate the amount of bone in children regardless of age, sex, or whether they are healthy or sick. The immediate challenge is to obtain valid interpretations of DXA bone measurements in pediatrics so that a subclinical deficiency in bone accrual can be identified accurately in "at risk" children. To this end, greater understanding of the DXA errors associated with variations in growth and development and the methods to correct for size bias and soft tissue distribution is needed.

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Low-Level, High-Frequency Mechanical Signals Enhance Musculoskeletal Development of Young Women With Low BMD

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ABSTRACT: The potential for brief periods of low-magnitude, high-frequency mechanical signals to enhance the musculoskeletal system was evaluated in young women with low BMD. Twelve months of this noninvasive signal, induced as whole body vibration for at least 2 minutes each day, increased bone and muscle mass in the axial skeleton and lower extremities compared with controls.

Introduction: The incidence of osteoporosis, a disease that manifests in the elderly, may be reduced by increasing peak bone mass in the young. Preliminary data indicate that extremely low-level mechanical signals are anabolic to bone tissue, and their ability to enhance bone and muscle mass in young women was investigated in this study.
Materials and Methods: A 12-month trial was conducted in 48 young women (15–20 years) with low BMD and a history of at least one skeletal fracture. One half of the subjects underwent brief (10 minutes requested), daily, low-level whole body vibration (30 Hz, 0.3*g*); the remaining women served as controls. Quantitative CT performed at baseline and at the end of study was used to establish changes in muscle and bone mass in the weight-bearing skeleton.
Results: Using an intention-to-treat (ITT) analysis, cancellous bone in the lumbar vertebrae and cortical bone in the femoral midshaft of the experimental group increased by 2.1% ($p = 0.025$) and 3.4% ($p < 0.001$), respectively, compared with 0.1% ($p = 0.74$) and 1.1% ($p = 0.14$), in controls. Increases in cancellous and cortical bone were 2.0% ($p = 0.06$) and 2.3% ($p = 0.04$) greater, respectively, in the experimental group compared with controls. Cross-sectional area of paraspinous musculature was 4.9% greater ($p = 0.002$) in the experimental group versus controls. When a per protocol analysis was considered, gains in both muscle and bone were strongly correlated to a threshold in compliance, where the benefit of the mechanical intervention compared with controls was realized once subjects used the device for at least 2 minute/day ($n = 18$), as reflected by a 3.9% increase in cancellous bone of the spine ($p = 0.007$), 2.9% increase in cortical bone of the femur ($p = 0.009$), and 7.2% increase in musculature of the spine ($p = 0.001$) compared with controls and low compliers ($n = 30$).
Conclusions: Short bouts of extremely low-level mechanical signals, several orders of magnitude below that associated with vigorous exercise, increased bone and muscle mass in the weight-bearing skeleton of young adult females with low BMD. Should these musculoskeletal enhancements be preserved through adulthood, this intervention may prove to be a deterrent to osteoporosis in the elderly.
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Key words: **osteoporosis, treatments, mechanical, loading, novel entities, osteopenia, frequency, bone, adaptation, muscle, anabolic, osteogenic, CT diagnostics, therapeutics**

INTRODUCTION

SUSCEPTIBILITY FOR LOW bone mass is present early in life, the amount of bone gained during adolescence is a main contributor to peak bone mass in the young adult, and peak bone mass in the young adult is a likely determinant of osteoporosis in the elderly.[1,2] Whereas research continues to identify means of reversing osteoporosis in the elderly, these data from children, adolescents, and young adults indicate that enhancing bone health early in life represents a viable means of deterring osteoporosis decades before it arises.[3] However, the benefits of early pharmacological interventions to prevent a disease that will not manifest for

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decades must be weighed against the possible complications of extended treatment.[4,5] To date, most interventions have focused on antiresorptive medications that inhibit the cellular processes of bone turnover,[6] yet, when prescribed as a decades-long prevention strategy, may compromise both bone quality[7] and viability.[8] As importantly, the critical roles of muscle strength and neuromuscular control in the reduction of falls and fractures fail to be addressed with interventions that specifically and exclusively targets bone.[9]

Considerable interest has, therefore, been placed on studying controllable environmental factors, such as physical exercise, which can promote bone and muscle gains during growth,[10] well before bone mass has reached its peak.[11,12] Maximizing the benefits of the mechanical regimen without putting the skeleton at risk creates a challenge to identify, and thus focus on, the anabolic components of the loading environment. A common perception of skeletal adaptation to exercise is that the mechanical loads must be great to augment bone mass, such that vigorous physical exercise will induce bone strains sufficient to cause microdamage and stimulate bone formation through the repair of damaged tissue.[13,14] In contrast to these large loads and the potential damage they may cause, extremely low-level, high-frequency strains on bone mass, similar to those caused by muscle contractibility during postural control,[15] have recently been shown to be anabolic to bone tissue.[16] Animal studies indicate that low-magnitude high-frequency strains, induced through vibration, can stimulate bone formation in weight-bearing regions of the skeleton.[17,18] Translating this potential to the clinic, preliminary evidence indicates such signals can effectively inhibit bone loss in postmenopausal women[19] and enhance bone acquisition in children with disabling conditions.[20]

Approximately one in three children suffer a bone fracture by the time they reach skeletal maturity.[21] Whereas strenuous physical activity and occupational hazards are key factors in the pathogenesis of these fractures, several studies indicate that teenagers who sustain fractures also have decreased bone mass.[22–25] Therefore, the use of low-level mechanical signals to strengthen bone in young subjects with low bone mass may be relevant not only to the treatment of existing skeletal fragility, but, by enhancing peak bone mass and retaining it through adulthood, reduce the risk of osteoporosis and fractures later in life. This study was designed to establish whether brief, daily exposure to extremely low-level mechanical stimuli was anabolic to musculoskeletal development in young females, 15–20 years of age, each with low BMD and who had already sustained a fracture. Considering that these young women are highly likely to achieve only a low peak bone mass and therefore may be at greater risk of osteoporosis later in life, it was projected that a nonpharmacologic enhancement of the musculoskeletal system early on, if retained, could help diminish this debilitating disease.

MATERIALS AND METHODS

The study design, protocol, and consent forms were reviewed and approved by the institutional review board at Childrens Hospital Los Angeles (CHLA) and The Surgeon General's Human Subjects Research Review Board, and all participants and the parents of those <18 years of age signed informed consent.

Study subjects

The subjects for this study were healthy white females 15–20 years of age, all of whom had previously sustained at least one fracture. An initial interview was conducted with the subjects and their parents to describe the purpose and the aims of the study and the tests that would be performed. Candidates for this study were excluded if they had a diagnosis of any underlying disease or chronic illness, if they had been ill for >2 weeks during the previous 6 months, if they had been admitted to the hospital at any time during the previous 3 years, or if they were taking any medications including oral contraceptives. Candidates who were pregnant, had ever been pregnant, or with an absence of menses for >4 consecutive months or two cycle lengths after establishing regular cycles were also excluded from the study.

All potential candidates underwent a physical examination to determine their general health, vital signs, and stage of sexual development. Only females who had completed puberty (Tanner stage V of sexual development) were considered eligible for this study.[26] Thereafter, height, sitting height, weight, and body mass index (BMI) were determined, and skeletal age was determined from roentgenograms of the left hand and wrist.[27] Females in whom the epiphyses of the phalanges and the metacarpals had not fused completely were excluded to avoid inclusion of subjects with constitutional delay of growth.

Using this approach, candidates were evaluated until 150 were enrolled. Subsequently, CT measures were obtained, and the 50 subjects with the lowest CT values for vertebral cancellous BMD (–1 SD below mean peak BMD values) were invited to participate in the intervention phase of this study.[28] These subjects were assigned to the mechanical intervention or the control group based on their home address, with the 25 subjects living closest to CHLA selected to participate in the mechanical intervention and the remaining 25 serving as controls. Subjects assigned to the control group did not participate in the mechanical intervention schedule, but underwent the same baseline and follow-up examinations as the subjects in the intervention group.

Dietary and physical activity assessments

Dietary and physical activity questionnaires were completed at baseline and 6 and 12 months. Nutritional status was assessed using written recall records of dietary intake.[29] To account for the possible confounding effect of calcium intake, all participants were provided with a daily dose of one tablet of fruit-flavored TUMS 500 (GlaxoSmithKline, Pittsburgh, PA, USA), consisting of 500 mg of elemental Ca as Ca carbonate/tablet, for 1 year. Compliance was maximized through weekly telephone contacts.

Levels of physical activity in all study participants were examined using a 7-day physical activity recall questionnaire at baseline, 6 months, and completion of the study.

Participants were asked to indicate the number of times in the past week they engaged in strenuous, moderate, and mild forms of physical activity for >15 minutes. Definitions of each type of physical activity, as well as several examples of sport types in each category, were provided so that subjects fully understood these terms. A total score was obtained by multiplying responses in each intensity category by values corresponding to multiples of resting energy expenditure and summing the products. Thus, this measure represents frequency, intensity, and duration elements of physical activity with a test–retest reliability coefficient of 0.81.[30,31]

CT measurements of bone and muscle mass

All participants were assessed by CT using the same scanner (Hilite Advantage; General Electric, Milwaukee, WI, USA) and the same mineral reference phantom for simultaneous calibration (CT-T bone densitometry package; General Electric), and all studies were performed by the same technologist. In the axial skeleton, identification of the sites to be scanned was performed with lateral scout views and measurements of the density of cancellous bone and the cross-sectional dimensions of the vertebral bodies were obtained at the first, second, and third lumbar vertebrae; these measures are a reflection of the tissue density of bone in milligrams per cubic centimeter. In the femur, location of the site to be scanned was determined by physical examination, and the cross-sectional area (mm^2) and cortical bone area (mm^2) at the midshaft of the bone were obtained. A critical consideration in any CT study,[31] the CVs for repeated CT measurements of vertebral cancellous BMD and vertebral body cross-sectional area and of cortical BMD, cortical bone area, and the cross-sectional area of the femur ranged between 0.6% and 1.5% at our facility.[32]

From the same CT cross-sectional images obtained at L_1, L_2, and L_3 and at the midshaft of the femur, the areas of paraspinous and quadriceps femoris muscles (mm^2) were determined. For the purpose of this study, paraspinous musculature was defined as the combined area of the iliopsoas, erector spinae, and quadratus lumborum muscles. At our facility, the CVs for repeated CT measurements of muscle in the thigh and trunk fell between 1% and 2%.[33]

The time required to complete CT scans in individual patients was ~10 minutes. CT measurements were obtained at 1.5 or 1.0 mm thickness, 80 kVp, 70 mAmp, and 2 s. Radiation was 100–150 mrems (10–15 mJ/kg) localized to the 10-mm-thick section of imaging in the midportions of the L_1, L_2, and L_3 vertebral bodies and the 1.5-mm-thick section of the midthigh. The effective radiation dose was ~10 mrem (0.10 mJ/kg), including that associated with the scout view.[34]

DXA determinations of bone and body composition

All participants were also assessed with the Hologic QDR4500 (General Electric) DXA scanner, and all studies were performed by the same technologist. BMC (g) and areal BMD (aBMD, g/cm^2) were measured for the total body and lumbar spine. In addition, total fat mass (kg) and total lean mass (kg) were determined from the total body scan. Precision for aBMD values of the total body and spine was 0.4% and 1.6%, respectively, and for total fat mass and total lean mass was 3.1% and 0.6%, respectively. Total body scans required <5 minutes and have a total body radiation exposure of 0.4 mrem, whereas spine scans were obtained in 30 s with a skin entrance exposure of 3.7 mrem.[29]

Mechanical stimulus intervention

The mechanical intervention device has been previously described in detail.[35] Briefly, to deliver low-level mechanical signals to the weight-bearing skeleton in a controlled manner, a small (36 × 36 × 9 cm) platform was designed to induce a vertical, sinusoidal acceleration. The top platen of the platform accelerated at 0.3*g*, peak to peak (1.0*g* = Earth's gravitational field = 9.8 m/s^2) and at a frequency of 30 Hz (cycles per second) through a low force (18N) coil actuator (model LA18–18; BEI, San Marcos, CA, USA). This acceleration is well below International Organization for Standardization (ISO) and Occupational Safety and Health Administration (OSHA) recommendations for human limits of vibration exposure.[36,37] Displacement of the top platen at 30 Hz, 0.3*g*, was <50 μm.

The intervention was performed after the installation of the mechanical devices in the homes of the young women. Subjects were instructed to stand on the platform for 10 minutes each day for 12 months. Each device was equipped with a built-in electronic monitoring system that automatically recorded the duration the device was used each day. Compliance was assessed through monthly calibrations and data downloading, as well as weekly telephone contacts.

Statistical analysis

Both an intention-to-treat (ITT) analysis, which included all experimental and control subjects who began the protocol at baseline, and a per protocol (PP) analysis, designed to exclude drop-outs and poor compliers, were performed. Statistical analysis was performed using Stata 8.0 (StataCorp, College Station, TX, USA) and SPSS 13.0 for Windows (Chicago, IL, USA). All values shown are presented as mean ± SD, unless otherwise stated. The sample size was determined a priori by anticipating a balanced study with a difference in vertebral cancellous BMD gains between experimental and control subjects of 4% over 12 months, assuming an enhanced response over that achieved in the spine when a 0.2*g*, 30-Hz signal was used in a group of postmenopausal women,[19] and values for cancellous BMD in the lowest quartile to be 178 ± 9 mg/cm^3.[1] A sample size of 25 subjects in each group resulted in a power of 0.80 with an α of 0.05.

In the ITT analysis, baseline characteristics were compared with a two-sample *t*-test. Paired *t*-tests evaluated changes in measurements over baseline, and an unpaired *t*-test was used to compare both actual changes as well as the relative (percentage) changes over time for the control and treatment groups. This evaluation is equivalent to a repeated-measures ANOVA, which was used to include baseline measures such as bone age or height as covariates. Multivariate ANOVA simultaneously compared various changes over time in the axial and appendicular skeleton.

TABLE 1. BASELINE MEASURES AND p VALUES FOR ANTHROPOMETRIC PARAMETERS, PHYSICAL ACTIVITY, AND CALCIUM INTAKE FOR THE CONTROL AND EXPERIMENTAL GROUPS (N = 24 IN EACH GROUP)

	Control	*Experimental*	*p*
Age (years)	17.6 ± 1.3	17.3 ± 1.5	0.45
Bone age (years)	17.4 ± 0.7	17.0 ± 1.0	0.12
Height (cm)	164.0 ± 6.1	160.8 ± 3.8	0.037
Weight (cm)	67.5 ± 15	63.3 ± 13.7	0.32
BMI (kg/m^2)	25.1 ± 5.5	24.5 ± 5.5	0.72
Physical exercise index (h/wk)	9.9 ± 9.0	11.3 ± 11	0.74
Inactivity index (h/wk)	8.9 ± 9.3	5.6 ± 3.9	0.11
Calcium intake (mg/day)	1138 ± 814	1354 ± 1251	0.48

The single significant difference in these baseline parameters was height, where controls were 3.2 cm taller (p = 0.037).

The PP analysis was designed to identify any dose:response relationship, in which efficacy of the device could be shown as dependent on compliance, or if a "threshold" response, similar to that observed in animal experiments, arose where once a given number of loading cycles was passed, additional loading provided no additional benefit to bone tissue.[38] In this posthoc analysis, the experimental cohort was subdivided into quartiles[19] to allow a comparison between the women who were the lowest 25% of compliers relative to those who fell between 25% and 50%, 50% and 75%, and 75% and 100%, representing those women who were closest to the requested 10 minute/day treatment regimen, and thus to determine if a minimal use for the device could be approximated.[20]

RESULTS

Of the 150 women who volunteered for the study, the 50 women with the lowest BMD were enrolled in the study. Two subjects, one in the experimental group and one in the control group, began the use of oral contraceptives between the time of enrollment and the start of protocol and were removed from the study before the start of protocol. Those women closest to the hospital were enrolled in the treatment arm of the study, and Table 1 shows the baseline characteristics of the control (N = 24) and treatment groups (N = 24). Despite a subject pooling based on the proximity of their residence to CHLA, the sole measure that was significantly different between groups at baseline was height; women in the control group were 1.8% taller than those in the experimental group (p = 0.037).

ITT analysis

Over the course of the 1-year study, experimental and control subjects showed identical increases in height (0.4%) and similar increases in weight (2.6% and 2.1%, respectively), BMI (1.9% and 1.4%, respectively), and calcium intake (42% and 36%, respectively), with no significant differences at follow-up in measures of physical activity or inactivity. There were no reported adverse reactions to the treatment.

Table 2 summarizes the results from the ITT analysis, with baseline and follow-up CT values for muscle and bone in the axial and appendicular skeleton presented for all control and experimental subjects (n = 24 in each group). Baseline values for the panel of musculoskeletal measures were not significantly different in the experimental group than those measured in the controls. Whereas significant increases were present at follow-up for all morphological traits in the experimental group, the only significant change observed in the control group was evident in the cross-sectional area of the femur.

Table 3 presents the absolute changes and percent changes for all women in each of the two groups. In the axial skeleton, significantly greater increases were evident in the absolute and/or percent change of paraspinous musculature of the experimental group over all controls, with 6.0% greater gains measured in the psoas ($p < 0.003$) and 4.4% in the erector spinae (p = 0.03). The spine had 2.0% more cancellous bone in the experimental than the control cohort (p = 0.06).

In the appendicular skeleton, experimental subjects had 2.3% greater increase than controls in femoral cortical bone area ($p < 0.04$; Fig. 1). Considering that the cross-sectional area defined by the periosteal envelope (femur cross-sectional area) was similar in the two groups (mean area increase in each cohort increased 0.1 cm^2; p = 0.25), the increase in bone area was achieved through apposition on the endosteal surface.

None of the baseline variables showed a significant correlation with any of the absolute or percent changes over the 12-month experimental period. As a result, p values changed insignificantly when any of these baseline characteristics were considered as covariates for the absolute and relative comparison between controls and experimental subjects.

Statistically significant differences between experimental and controls were also found when the changes from all outcome variables were analyzed as a vector of observation using a multivariate repeated-measure ANOVA; this was true whether the analysis was based on absolute change or percent changes, with or without covariates ($p < 0.05$). When separated into two anatomical regions, significant differences were observed for the axial, but not for the appendicular, skeleton.

PP analysis

Compliance in the 24 women in the experimental group was highly variable, ranging from 1% to 100%, with a mean compliance of 130.3 ± 92.1 minutes/month or 4.3 minutes/day (Fig. 2A). A posthoc, PP analysis was used to determine whether there was a dose:response benefit of treatment duration or whether a compliance threshold existed, beyond which exposure to mechanical intervention no longer provided additional benefit. The experimental cohort was stratified into quartiles according to their percent compliance, with the bottom quartile including compliance values between 1% and 13% (n = 6), the second lowest quartile of compliance between 21% and 39% (n = 6), the second highest quartile fell between 41% and 71% of compliance (n = 6), and the quartile with the highest compliance was between 77% and 100% of compliance (n = 6).

TABLE 2. BASELINE AND 1-YEAR CT MEASURES AND p VALUES FOR SPECIFIC MUSCULOSKELETAL REGIONS WITHIN THE AXIAL AND APPENDICULAR SKELETON FOR BOTH CONTROL AND EXPERIMENTAL GROUPS (N = 24 IN EACH GROUP)

	Control			*Experimental*		
	Baseline	*1 year*	*p*	*Baseline*	*1 year*	*p*
Axial						
Total paraspinous musculature (cm^2)	181.6 ± 26	182.8 ± 27	0.52	167.5 ± 29	177.5 ± 31	<0.001
Psoas (cm^2)	48.7 ± 8.2	48.7 ± 7.70	0.99	45.0 ± 9.5	48.0 ± 10.9	<0.001
Quadratus lumborum (cm^2)	20.9 ± 5.9	21.9 ± 6.70	0.08	19.1 ± 3.6	21.2 ± 4.3	<0.001
Erector spinae (cm^2)	112.0 ± 15.0	112.2 ± 15.0	0.89	103.4 ± 21	108.3 ± 21	0.03
Spine cancellous BMD (mg/cm^3)	171.3 ± 17.1	171.5 ± 14.9	0.93	164.8 ± 25	168.6 ± 25	0.03
Appendicular						
Quadriceps femoris muscle (cm^2)	112.0 ± 16.0	114.6 ± 14.0	0.14	104.4 ± 13	108.5 ± 15	<0.001
Femur cross-sectional area (cm^2)	5.12 ± 0.77	5.17 ± 0.82	0.05	4.82 ± 0.53	4.92 ± 0.52	0.003
Femur cortical bone area (cm^2)	4.18 ± 0.51	4.24 ± 0.58	0.14	3.96 ± 0.43	4.10 ± 0.42	<0.001

The only significant change in the control group was in cross-sectional area of the femur (p = 0.05). In contrast, there were significant changes measured in each region of the axial and appendicular skeleton of the experimental group.

TABLE 3. AFTER THE 1-YEAR EXPERIMENTAL PROTOCOL, ABSOLUTE AND PERCENT CHANGE IN CT MEASURES OF SPECIFIC MUSCULOSKELETAL REGIONS OF THE AXIAL AND APPENDICULAR SKELETON FOR ALL THE WOMEN IN THE CONTROL AND EXPERIMENTAL GROUPS (N = 24 IN EACH GROUP)

	Absolute change			*Percent change*		
	Control	*Experimental*	*p*	*Control*	*Experimental*	*p*
Axial						
Total paraspinous musculature (cm^2)	1.2 ± 9.0	10.1 ± 12.5	0.007	0.5 ± 5.0	5.4 ± 6.9	0.002
Psoas (cm^2)	0.0 ± 2.9	3.1 ± 3.5	0.002	–0.1 ± 0.1	5.9 ± 6.7	0.003
Quadratus lumborum (cm^2)	1.0 ± 2.7	2.2 ± 2.6	0.16	3.0 ± 14.7	9.0 ± 11.7	0.17
Erector spinae (cm^2)	0.2 ± 5.6	5.3 ± 11.0	0.05	–0.1 ± 0.9	4.3 ± 8.8	0.03
Spine cancellous BMD (mg/cm^3)	0.1 ± 7.7	3.8 ± 7.7	0.11	0.1 ± 4.5	2.1 ± 4.9	0.06
Appendicular						
Quadriceps femoris area (cm^2)	2.6 ± 8.4	4.1 ± 4.5	0.45	2.2 ± 2.7	3.6 ± 3.6	0.36
Femur cross-sectional area (cm^2)	0.1 ± 0.1	0.1 ± 0.2	0.25	0.9 ± 2.2	1.9 ± 3.4	0.28
Femur cortical bone area (cm^2)	0.05 ± 0.17	0.14 ± 0.15	0.08	1.1 ± 3.7	3.4 ± 3.7	0.04

p values reflecting the difference between the control and experimental groups are also given.

A dose effect was evident in the erector spinae muscle, providing a first indication of a significant increase in muscle mass achieved at 20% compliance (2 minutes/day; Fig. 2B). When assessed by the responsivity of specific quartiles of compliance, clear threshold characteristics were observed in a number of musculoskeletal sites, with the lowest quartile failing to respond at all to the intervention, and the three highest quartiles being very similar in their responses (Fig. 3). Given the nonresponsivity of those in the lowest quartile of compliance, these subjects were pooled with controls. Moving these low compliers into the control groups further reduced the small differences in baseline characteristics between control and experimental subjects, including the p value for the difference in height from <0.05 to 0.8.

As summarized in Table 4, women who used the intervention at least 2 minutes/day (n = 18) showed significant increases over the group pooling controls and those in the lowest quartile of compliance (n = 30). Figure 4 shows the differences between groups and includes an 8.3% greater cross-sectional area of the erector spinae musculature in highly compliant women over controls and low compliers (p = 0.006), a 5.2% increase in the cross-sectional area of

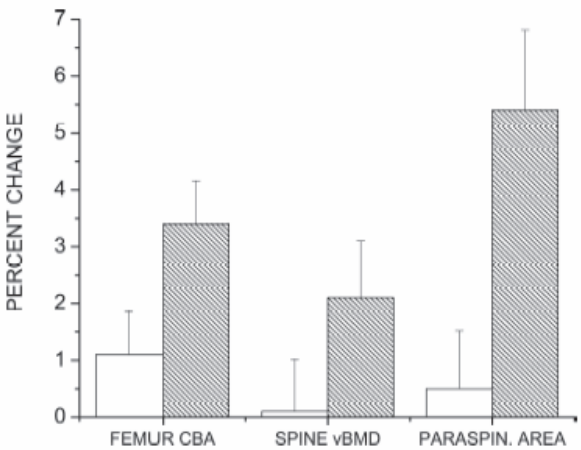

FIG. 1. Percent change (mean ± SE) occurring over the 1-year protocol, from both the control (white bars) and experimental (striped bars) subjects, using an intention-to-treat analysis and therefore including all 24 subjects who began the protocol in each group. The graph presents the CT data from the cortical bone area of the femur (p = 0.04), the cancellous BMD of the spine (p = 0.06), and the total paraspinous musculature (p = 0.002).

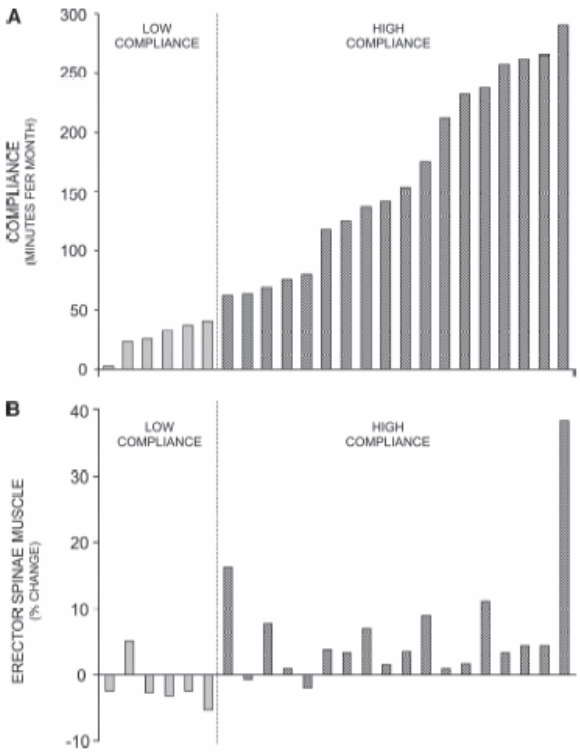

FIG. 2. (A) Compliance for each of the 24 subjects in the experimental group, as expressed in minutes per month. Each subject was requested to use the device for 10 minutes/day, such that 300 minutes/month would represent 100% compliance. Experimental subjects are represented either as those who used the device <20% of the allotted time (stippled bars) and are indicated as low compliance ($N = 6$) or those who used the device for >20% of the time (striped bars) and are indicated as high compliance ($N = 18$). (B) Percent change in the cross-sectional area of the erector spinae muscle of each experimental subject, as related to their compliance (above).

the psoas ($p = 0.02$), 7.2% greater mass in the total paraspinous musculature of high compliers ($p = 0.001$), a 3.9% greater density in the cancellous bone of the spine ($p = 0.007$), and a 2.9% greater cortical bone area in the femur ($p = 0.009$). No significant differences were observed in the musculature of the femur or in the cross-sectional area—in contrast to cortical bone area—of the femur.

DXA

Baseline and follow-up DXA values are shown in Table 5. Mean values for spine BMC and aBMD and for total body BMC were significantly higher in both groups at follow-up. In addition, in the experimental group, values for total body aBMD were higher after the intervention. There were, however, no significant differences between groups in the absolute and/or percent change for any of these DXA measures of bone and body composition (Table 6).

DISCUSSION

The data from this study indicate that the formation of bone and muscle can be enhanced in young women with low BMD by short daily exposure to extremely low-magnitude mechanical signals. It is presumed that the physiologic basis of these exogenous signals is that they serve to amplify the spectral content of endogenous muscle contractibility that are projected to the skeleton during even passive activities such as standing.[15] That the controls and women with low compliance significantly increased only a single musculoskeletal parameter over the course of a year, whereas there were significant increases in each musculoskeletal parameter in the experimental group, emphasizes that the skeleton is readily responsive to mechanical signals, and they do not need to be "big" to be anabolic.

This study supports the premise that mechanical signals, orders of magnitude below that which might cause damage to the bone matrix,[39] can enhance musculoskeletal development. The ITT analysis revealed that 1 year of these mechanical signals increased cancellous bone in the axial skeleton and cortical bone in the appendicular skeleton by 2.0% and 2.3% over controls, respectively. Simultaneous to these gains in bone, low-magnitude high-frequency mechanical signals significantly increased muscle mass; close to a 5% greater increase in cross-sectional area of paraspinous musculature was detected in women in the intervention group compared with controls.

As with any intervention, it is important to emphasize that the treatment will only be effective if it is actually used.[40] The PP analysis revealed a direct dependence of efficacy on compliance; women using the vibration system at least 2 minutes/day realized a benefit of the intervention through gains in cancellous and cortical bone and paraspinous musculature as opposed to women who used it <2 minutes/day, who showed no changes in their skeletal parameters that were different than measured in controls. In those women who used the device at least 2 minutes/day, increases reached 7.2% in the spinal musculature, 3.9% in the cancellous bone of the spine, and 2.9% in the cortical bone of the femur compared with controls pooled with poor compliers. Once the 2-minute duration was surpassed, women, even in the highest quartile of compliance, reaped no additional benefit of use, suggesting that a biologic response was triggered rather than accumulated.[38]

The mechanism(s) by which extremely low-level mechanical signals can enhance the musculoskeletal system are currently unknown.[41] The physical basis of translating low-level mechanical signals into a biological response could result from an amplification system achieved through fluid movement through the canalicular system of osteocytes[42] and promoted by the interdependence of fluid pressure and frequency.[43] From a biologic perspective, the enhanced skeletal mass could result from alterations in the transcriptional control of the bone tissue either by upregulating genes involved in bone formation, downregulating genes involved in the resorption of bone, or both.[44] Certainly, it is possible that adaptation of the musculoskeletal system to exogenous signals is preferentially sensitive to higher frequency signals, similar to other physiologic systems designed to monitor "exogenous stimuli," such as vi-

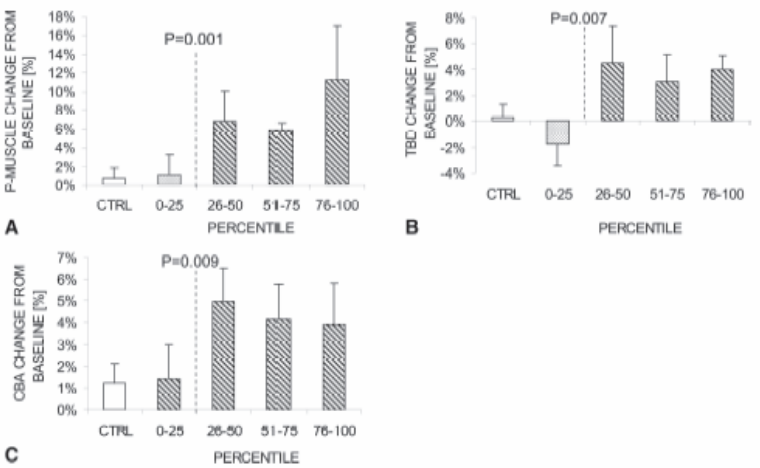

FIG. 3. Percent change (mean ± SE) measured over the 1-year period for (A) paraspinous musculature, (B) vertebral cancellous BMD, and (C) femoral cortical area in control subjects (white bars; $N = 24$) compared with experimental subjects in each of the compliance quartiles ($N = 6$ each). p values reflect comparison of subjects pooled from the three top compliance quartiles (compliance >20%) to the pooled low compliance (<20% compliance) plus the control group. Note very little change was measured in either the controls or the quartile representing the lowest compliers over the 1-year period, whereas the anabolic response to the mechanical signal did not increase beyond the 2-minute "threshold," implying a triggered response of bone to mechanical signals rather than an accumulated dose:response adaptation.

TABLE 4. USING A PER PROTOCOL ANALYSIS, SUBJECTS ($N = 6$) WITHIN THE LOWEST QUARTILE OF COMPLIANCE WERE POOLED WITH CONTROLS (CONTROLS + POOR COMPLIERS: TOTAL $N = 30$) AND COMPARED WITH THE ABSOLUTE AND PERCENT CHANGES MEASURED FROM CT IN THE SUBJECTS IN THE THREE HIGHEST QUARTILES OF COMPLIANCE (HIGH COMPLIERS: $N = 18$)

	Absolute change			*Percent change*		
	Control + poor compliers	*High compliers*	*p*	*Control + poor compliers*	*High compliers*	*p*
Axial						
Total paraspinous musculature (cm^2)	1.4 ± 8.9	12.6 ± 12.6	0.001	0.8 ± 5.1	8.0 ± 9.1	0.001
Psoas (cm^2)	0.6 ± 3.6	3.1 ± 2.8	0.01	1.6 ± 8.2	6.8 ± 6.0	0.02
Quadratus lumborum (cm^2)	1.1 ± 2.5	2.4 ± 2.7	0.11	5.4 ± 13.7	13.4 ± 15.0	0.07
Erector spinae (cm^2)	−0.3 ± 5.3	7.1 ± 10.4	0.002	−0.2 ± 4.7	8.1 ± 14.5	0.006
Spine cancellous BMD (mg/cm^3)	−0.4 ± 7.4	5.9 ± 7.2	0.006	−0.1 ± 4.5	3.8 ± 4.9	0.007
Appendicular						
Quadriceps femoris area (cm^2)	3.0 ± 7.8	4.0 ± 4.5	0.59	3.0 ± 6.8	3.9 ± 4.2	0.63
Femur cross-sectional area (cm^2)	0.05 ± 0.12	0.12 ± 0.16	0.10	1.0 ± 2.2	2.4 ± 3.7	0.12
Femur cortical bone area (cm^2)	0.05 ± 0.17	0.17 ± 0.13	0.02	1.3 ± 3.9	4.3 ± 3.6	0.009

Highly significant differences were observed in several regions of the spine musculature, as well as the cancellous bone of the spine and cortical bone area of the hip, whereas musculature around the femur and cross-sectional area of the femur were not significantly different between groups.

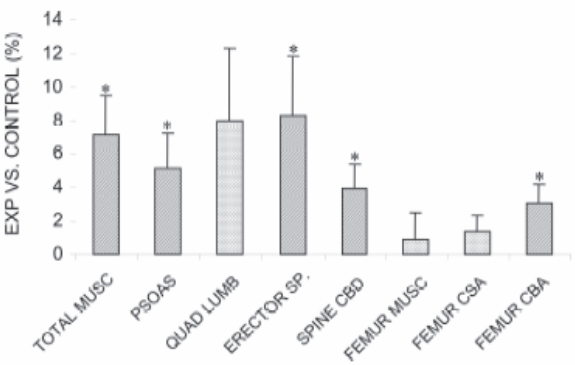

FIG. 4. Difference in the change (mean ± SE) measured over the 1-year period for the those who used the device for >2 minutes/day compared with the controls pooled with the women in the lowest quartile of compliance. Each parameter evaluated, with the exception of musculature around the femur and femoral cross-sectional area, showed that the experimental group benefited significantly (*) from the mechanical intervention.

sion (color), hearing (tone), and tactile sense (pressure), and that these external signals are processed within specific windows of sensitivity and begin to shut down when the signal becomes too bright, too loud, or too heavy.

The physical and biologic mechanisms that control the adaptation of bone to its loading environment are complex[45] and involve the interaction of pathways mediated through gravity, muscle contractions, and physical activity, as well as a genetic component that defines the musculoskeletal system's susceptibility to mechanical signals.[46] Whereas the strain signals in this study fell well below those that are imposed on the skeleton by vigorous exercise,[47] they were significantly more robust than those experienced during minimal activities of daily life.[48] These extremely low-level strain magnitudes are intended to augment those mechanical signals that arise through muscle contractions during passive activities, such as maintaining posture, whereas remaining orders of magnitude below those strain levels may cause microdamage to bone tissue.[39,47] These data also support the proposed interdependence of the

TABLE 5. BASELINE AND FOLLOW-UP DXA VALUES FOR SPECIFIC REGIONS OF THE MUSCULOSKELETAL SYSTEM AND WHOLE BODY MEASURES FOR BOTH CONTROL AND EXPERIMENTAL SUBJECTS (N = 24 IN EACH GROUP)

	Control			*Experimental*		
	Baseline	*1 year*	*p*	*Baseline*	*1 year*	*p*
Spine BMC (g)	56.1 ± 8.4	58.3 ± 7.8	<0.001	50.7 ± 6.1	52.7 ± 6.0	<0.001
Spine aBMD (g/cm^2)	1.02 ± 0.1	1.04 ± 0.1	0.003	0.95 ± 0.1	0.98 ± 0.8	0.002
Whole body BMC (g)	1614 ± 258	1676 ± 270	<0.001	1481 ± 184	1535 ± 177	<0.001
Whole body aBMD (g/cm^2)	0.98 ± 0.08	0.99 ± 0.07	0.15	0.94 ± 0.06	0.95 ± 0.06	0.05
Trunk lean mass (kg)	19.8 ± 2.7	20.0 ± 2.5	0.34	18.4 ± 2.4	18.9 ± 2.6	0.07
Total lean mass (kg)	40.1 ± 5.9	40.8 ± 5.6	0.06	37.8 ± 5.2	38.6 ± 5.8	0.15

Whereas significant changes were measured in several parameters within each group, the magnitude of these changes were not significantly different between groups (Table 6).

TABLE 6. ABSOLUTE AND PERCENT CHANGE IN DXA MEASURES FOR WOMEN IN THE CONTROL AND EXPERIMENTAL GROUPS (N = 24 IN EACH GROUP)

	Absolute change			*Percent change*		
	Control	*Experimental*	*p*	*Control*	*Experimental*	*p*
Spine BMC (g)	2.14 ± 2.18	2.07 ± 1.97	0.91	3.82 ± 4.07	3.93 ± 3.84	0.92
Spine aBMD (g/cm^2)	0.02 ± 0.03	0.02 ± 0.03	0.99	2.11 ± 3.22	2.25 ± 3.19	0.88
Whole Body BMC (g)	59.5 ± 57.8	53.5 ± 53.8	0.71	3.45 ± 3.45	3.52 ± 3.34	0.94
Whole body aBMD (g/cm^2)	0.01 ± 0.02	0.01 ± 0.02	0.57	0.65 ± 1.87	0.96 ± 2.29	0.61
Trunk lean mass (g)	214 ± 1058	460 ± 1174	0.45	1.06 ± 4.93	2.19 ± 6.03	0.49
Total lean mass (g)	702 ± 1704	754 ± 2456	0.93	1.75 ± 4.07	1.61 ± 5.95	0.93

No significant differences between control and experimental subjects were identified.

musculoskeletal "system," in that conditions such as sarcopenia[9] and the deterioration of the spectral content of muscle contraction[15] would diminish key regulatory components to the skeleton and thus conspire to contribute to the etiology of osteopenia.

The anabolic effects of the intervention on muscle and bone were present even after accounting for body weight, despite previous suggestions that low-magnitude mechanical stimulation would be most beneficial in subjects with lesser body weight.[19] Whereas it is entirely possible that the responsivity of the experimental group was caused by the signal magnitude being 50% higher than the study on postmenopausal women (0.3g versus 0.2g), it may also be that all the women in this study began with low BMD, and thus the entire cohort was more sensitive to the mechanical signals. This can be considered in the context that mice with low BMD are more sensitive to the high-frequency mechanical signal than mice with dense bone,[49] but whether this is by virtue of the signal being greater in lighter bones or because bones more prone to disuse osteoporosis are, in turn, more sensitive to mechanically based augmentation, is not yet clear. It is also possible that the women in this study, like the children with disabling conditions,[20] were responsive because they were young, and that the ability to proliferate and differentiate pre-osteoblasts into bone-producing cells is more readily achieved in younger organisms.[50]

The use of CT to obtain measures of muscle and bone in the appendicular and axial skeleton provided unique insight into the means by which the low-level mechanical signal worked and helped to identify the specific tissues and anatomic compartments that it influenced. In contrast, DXA cannot fully correct for errors associated with changes in body and skeletal size[29,31] and does not allow for the independent assessment of muscle mass from other lean tissues.[51] Along these lines, it is noteworthy that, in this study, CT helped identify significant differences in bone and in muscle between control and experimental subjects, which were not evident with DXA. For example, the use of CT showed that the experimental group realized a significant increase in the cross-sectional area of paraspinous musculature compared with controls, thus indicating a benefit of the mechanical intervention beyond that specific to bone. These data suggest that mechanical signals have the potential to influence both bone and muscle, and considering the importance of muscle function to the incidence of falls and fall-related injuries, indicates that this intervention may be useful in reducing osteoporosis risk factors for fracture that drug therapies fail to address.[52]

There are several limitations in this study, and the results must be addressed and interpreted in context with its design. First, it is important to emphasize that this was not a randomized study because, by design, subjects were assigned to either the mechanical intervention or the control group based on their residential address; participants living closer to CHLA were assigned to the mechanical intervention to facilitate equipment maintenance, calibration, and data downloading. Whereas randomization did not occur, the baseline measures identified only height to be significantly different between the experimental and control subjects, and considering height as a covariate did not alter the statistical outcomes. Additionally, the subjects were not re-

cruited from the community at large, but were selected from young white female volunteers with low BMD and a history of fracture(s). It should be realized, however, that the intent of this study was specifically to determine if the skeletons of young women with low BMD could be enhanced with low-level mechanical signals, not if any given individual could realize a benefit from treatment. It is entirely possible that our results may not apply to subjects with denser bones, older (or younger) women, other ethnic groups, or men. Similarly, our findings apply to a specific type of mechanical stimulus, and it is likely that other types of vibration loading may result in varying effects on bone mass. Indeed, a recent 8-month study in healthy young adults found no effect of brief (4 minute), three to five times per week, high-magnitude (8*g*) whole body vibration training on bone mass, although this stimulus improved vertical jump height.[53] The differing study populations, the assays used to measure musculoskeletal response, and the wide disparity in magnitude of the mechanical stimulation (0.3*g* here, 8.0*g* there) are likely explanations for the discrepancy between results. It is also possible that musculoskeletal tissues of healthier subjects with stronger bones may not be as responsive to this range of loading. Data from animal studies suggest an individualized set point to mechanical signals; the anabolic potential of mechanical stimulus is greater in inbred mice strains with low BMD, whereas strains with high BMD have a lesser response to mechanical signals.[38]

It is important to emphasize that this study also does not address what will happen to the bone and muscle gains achieved in the mechanically stimulated cohort once treatment ceases. As with other anabolic interventions, such as PTH,[54] it is possible that gains in bone will be lost once treatment has stopped, and that other strategies (e.g., antiresorptive drugs, exercise) will have to be implemented to curb progressive deterioration. Whether gains realized even by exercise are preserved over time is controversial,[55] with evidence indicating that the bone accretion achieved through high-impact loading in premenopausal[56,57] and elderly[58] women is readily maintained after cessation of exercise, whereas other studies indicate that bone gains achieved in premenopausal women are at risk once exercise stops.[59] Extrapolating from the increases in muscle mass that parallel the gains in bone shown in this study, there is some possibility that the additional mechanical challenge derived from the muscle to the bone will contribute to the retention of the skeletal tissue even in the absence of the anabolic surrogate provided by the low-magnitude vibration.

At least 20% of the variance in bone mass is caused by controllable environmental factors, such as physical activity.[60] Unfortunately, exercise interventions have not proven overtly effective in the elderly because of difficulties with long-term compliance, a decline in the adaptive response to load bearing with aging,[61] and an increased risk of injury during vigorous exercise.[62] In contrast, enhancing the musculoskeletal system during early adulthood, and thus raising the peak bone and muscle mass as an adult, may serve to mitigate the consequences of their inevitable age-related decline in strength and integrity.[12] This is particularly true for adolescents with fractures, because they are at greater risk of decreased bone mass after puberty.[63] This study suggests that noninvasive mechanical loading, induced orders of magnitude below that that associated with exercise, could represent a unique means of augmenting the musculoskeletal system, and perhaps reducing bone fragility. That these signals seem to enhance both bone and muscle also suggest that the mechanical modality addresses risk factors for osteoporosis beyond "simply" bone quantity and quality. Moreover, it seems that these low-intensity mechanical signals incorporate many aspects of the complex remodeling cycle, enhancing bone formation while suppressing bone resorption.[64] Many questions remain as to whether the musculoskeletal benefits observed in this study will persist over time or whether such an intervention will ultimately reduce falls and/or fractures. Certainly, such information will be of great value in evaluating the potential of a nondrug measure for the prevention of postmenopausal osteoporosis decades before it occurs.

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Fat Mass is Not Beneficial to Bone in Adolescents and Young Adults

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ABSTRACT

CONTEXT
While muscle mass is beneficial to bone, studies on the effect of fat mass on bone have yielded conflicting results.

OBJECTIVE
To assess the relations between lean and fat mass and bone structure.

DESIGN
Cross-sectional.

SETTING
General community.

SUBJECTS
Three hundred healthy sexually mature adolescents and young adults (150 males and 150 females) between the ages of 13 and 21.

MAIN OUTCOME MEASURE
We investigated the relations between dual-energy x-ray absorptiometry (DXA) measures of total body fat and lean mass and bone values obtained with DXA (legs and lumbar spine bone mineral density and bone mineral content) and computed tomography (CT) (cross-sectional and cortical bone areas of the femurs and cross-sectional area and cancellous bone density of the vertebrae).

RESULTS
Simple and multiple linear regression analyses showed significant positive relations between DXA lean mass and all CT and DXA measures of bone in the axial and appendicular skeletons (all P's <0.005). In contrast, while Pearson correlations between DXA measures of fat mass and bone parameters were generally positive, multiple regression analyses showed that fat mass, after accounting for lean mass, trunk height/leg length had a negative, or no, correlation with CT and DXA values for bone.

CONCLUSIONS
Our findings provide compelling evidence that, despite increased mechanical loading and independent of lean mass, adipose tissue is not beneficial to bone structure.

INTRODUCTION

Increased fat during adolescence is a major public health concern, is associated with the metabolic syndrome, and is a risk factor for many common adult conditions, such as cardiovascular disease, diabetes, hypertension and cancer (1-3). However, most, but not all, studies examining the possible relations between fat mass and bone mass have found a positive association between these two tissues, regardless of age (4-9). Indeed, available data suggest that increased fat enhances bone mass and may protect against osteoporosis in both children and adults (9-12). This positive fat-bone relation is credited not only to stresses from mechanical loading, but also to the metabolic effects of bone-active hormones secreted or regulated by adipocytes (13). Leptin, a satiety-regulating hormone that is produced by adipocytes, increases the proliferation and differentiation of osteoblasts in adult patients (14). Additionally, aromatization of androgen to estrogen by fatty tissue results in reduced osteoclast activity and possibly increased bone mass in children (13). In contrast, two studies in females from childhood to young adulthood reported fat mass to be negatively associated with bone mass (8, 15).

Discrepancies in the results from previous studies assessing the relation between fat and bone may be related to differences in the cohorts studied and to the use of dual-energy x-ray absorptiometry (DXA) to simultaneously obtain fat and bone measures. While DXA allows for accurate determinations of body fat and lean mass, DXA bone values are influenced by the amount and distribution of fatty tissues around the bone (16). In this investigation, the potentially confounding effects of age, pubertal stage, gender and ethnicity, were controlled by only enrolling white sexually mature males and females. Additionally, to account for the

possible influence of soft tissues on bone measurements, the effects of fat and lean mass on bone were assessed by both DXA and computed tomography (CT).

METHODS

Subjects

The institutional review board for clinical investigations at Childrens Hospital Los Angeles approved the investigational protocol, and informed consent was obtained from all parents and/or subjects. A total of 300 healthy white teenagers and young adults (150 males and 150 females) between the ages of 13 and 21 years were recruited from schools of Los Angeles County and enrolled in this study.

Study subjects had no known diagnosis of any chronic illness, no history of medical disorders resulting in a period of illness that interrupted their usual physical activity and/or nutritional status for more than one month in the two years prior to enrollment, no intake of any medications, vitamin preparations, or calcium supplements within the previous six months, and no hospitalization since birth.

All eligible participants underwent a physical examination by a pediatrician. Measurements of weight were obtained to the nearest 0.1 kg, using the Scale-Tronix (Scale-Tronix, Inc, Wheaton, Ill) and measurements of height and trunk height were obtained to the nearest 0.1 cm, using the Harpenden stadiometer (Holtain Ltd, Crymmych, Wales). For the purposes of this study, leg length was defined as the difference between total height and trunk height. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Tanner stage of sexual development was assessed based on breast development in females and testicular size in males (17); only subjects who had achieved sexual maturity (Tanner 5) were included in this study. Skeletal maturation was assessed by the method of Greulich and Pyle from radiographs of the

left hand and wrist (18), and those in whom skeletal age differed from chronological age by more than two years were excluded from further evaluation.

Fat, Lean and Bone Measurements

Measurements of fat and lean mass of the total body and bone mineral content (BMC) and bone mineral density (BMD) of the first three lumbar vertebrae were obtained using a fan-beam DXA densitometer (Delphi W; Hologic, Inc, Waltham, MA) in array mode and were analyzed with the manufacturer's software. The coefficients of variation for these DXA measurements have been reported to range from 0.7 to 1.7% (19, 20). The time required for the procedure was approximately six minutes and the radiation exposure was negligible.

On the same day and by the same technologist, CT bone measurements using the same scanner (CT Highlite Advantage; General Electric Co, Milwaukee, WI) and the same mineral reference phantom (CT-T bone densitometry package; General Electric Co, Milwaukee, WI) were obtained. For this study, in the appendicular skeleton, measurements of cross-sectional area (CSA) and cortical bone area (CBA) were acquired at the midshafts of the femurs. In the axial skeleton, measurements of cross-sectional area (CSA) and cancellous bone density (CBD) were obtained at the midportions of the first three lumbar vertebral bodies. Measurements of CBD in the axial skeleton represent the tissue density of bone and are the correlates of measures of CBA in the appendicular skeleton. The coefficients of variation for these CT measurements in young adults are between 0.6-1.5% (19). The time required for this procedure was approximately 10 minutes, and the effective radiation dose was approximately 8 mrem (21).

Power Calculations and Statistical Analysis

Previous studies indicate that weight explains approximately 80% of the variance in the cross-sectional dimensions at the midshaft of the femur after age, pubertal status, gender and ethnicity are taken into account (22). Based on these data, an n of 150 males and 150 females was deemed sufficient to allow the detection of a 2% variance with a greater than 80% power. The data were analyzed using simple linear regression and multivariate analyses.

RESULTS

Age, anthropometric parameters, DXA measures and CT values in females and males are described in Table 1. Vertebral CSA and BMC, femoral CSA and CBA, legs BMC and BMD, height and total lean mass were significantly higher in males (P's < 0.0001), while measurements of total body fat were higher in females (P < 0.0001). Based on current age- and gender-specific CDC reference standards, the BMI of 24% of the females and of 19% of the males was between the 85th and 95th percentiles, and of 12% of the females and of 15% of the males was greater than the 95th percentile.

Overall, moderate correlations were observed between lean and fat mass (r's = .71 and .49 for females and males, respectively; both P's < 0.0001). However, in subjects with BMI's >95th percentile, the associations were weaker or not significant [r's = .52 (P = .019) and .25 (P = .25) for females and males, respectively].

Measures of bone by CT and DXA were significantly correlated (r's between .28 and .89; Table 2). Regardless of technique, simple linear regressions demonstrated positive associations between measures for bone and values for lean mass in both males and females; the weakest between CT measures of CBD and lean mass (Figures 1 & 2 and Table 3). In females, measures of fat mass also correlated with all DXA and CT bone parameters, while, in males, these relations were weaker or nonexistent (Figures 1 & 2 and Table 3).

Multiple regression analysis of the independent effects of lean and fat mass on bone obtained after adjusting for leg length or trunkal height confirmed the strong positive effect of lean mass

on all bone parameters (Tables 4 & 5). In contrast, fat mass had a negative, or no, relation to measures of bone. In males, all DXA measurements and CT measures of vertebral CBD and femoral CBA were negatively related to fat mass, while the cross-sectional areas of the vertebral body and the femur did not enter into the model. In females, there were no associations between bone and fat determinations, with the exception of a negative relation between DXA leg BMD and fat mass.

DISCUSSION

The findings of this study corroborate previous studies indicating that, regardless of age or gender, lean mass has a strong positive influence on bone mass in the appendicular and axial skeletons (23-25). In contrast, we found that, after taking lean mass into account, measures of body fat had an inverse, or no, relation with parameters related to the structure and strength of bone. These findings are consistent with previous reports showing fat mass to be negatively associated with bone mass (8, 26) and those suggesting that bone strength is primarily determined by dynamic loads from muscle force, not static loads, such as fat mass (25). They, however, disagree with the contention for a beneficial effect of fat mass on bone and investigations suggesting that fat mass is an even stronger predictor than lean mass of bone density (4, 7, 27).

Overall, analyses using fat mass revealed that the negative contribution of adipose tissue offset its potential benefit as a mechanical load. The basis for the negative effect of fat on bone observed in this study is unknown. However, adipose tissue, once considered a metabolically passive fuel depot for energy substrate and insulation, has recently become apparent as a metabolically active tissue. It secretes multiple proteins (collectively called adipokines) into circulation, which play important roles in the modulation of various biological functions. Further studies are needed to elucidate the role of adipokines and other adipose-modulated biochemical signals as potential mediators of bone structure.

Regardless of the mechanisms involved in the fat-bone association, a link between these tissues is suggested by recent studies demonstrating that osteoblasts and adipocytes originate from the

same mesenchymal stem cells. These stem cells, through alternative activation of reciprocal transcriptional programs, differentiate into either cell lineage in a mutually exclusive way (28). In bone marrow, this could lead to a reciprocal relation between fat and bone, depending on the local milieu. The balance between osteoblast and adipocyte differentiation could be disrupted by environmental factors; decreased bone formation accompanied by increased adipogenesis occurs with immobility, whereas the opposite is associated with increased weight-bearing exercise (29).

The relatively large number of well-characterized subjects and the use of two techniques for the accurate and independent assessment of the contributions of lean and fat tissues on bone structure are major strengths of this study. Contrary to our notion that discrepancies among previous investigations were a reflection of the influence of soft tissues on DXA bone determinations, we found similar results regardless of the technique used. There are several limitations in this study, including its cross-sectional design and the inability to extrapolate our findings to other racial groups or elderly subjects. Future studies are needed to determine whether the deleterious effects of fat on vertebral and femoral bone in young healthy white subjects can be extended to other cohorts.

In conclusion, the pervasive negative health consequences of obesity involve many organ systems, and medical subspecialties, as well as a large proportion of the population. However, despite the dire repercussions of obesity, the traditional paradigm suggests that adiposity is beneficial to the skeleton and could protect against osteoporosis. Our findings challenge this widely held view and provide compelling evidence that despite increased mechanical loading, adipose tissue is not beneficial to bone structure in young men and women.

FIGURE LEGENDS

Figure 1. Relations between total lean mass and vertebral CSA (upper line), femoral CSA (middle line) and CBA (lower line) in 150 females (A) and 150 males (B), and between total fat and vertebral CSA (upper line), femoral CSA (middle line) and CBA (lower line) in 150 females (C) and 150 males (D).

Figure 2. Relations between vertebral CBD and lean mass (A) and fat mass (B) in 150 females (thin lines) and 150 males (thick lines).

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Table 1. Age, anthropometric characteristics, bone, lean and fat measurements in 150 females and 150 males

	Females	Males
	Mean ± SD (Range)	Mean ± SD (Range)
Age (yrs)	17.0 ± 1.7 (13.1 to 20.9)	17.4 ± 1.6 (14.0 to 21.0)
Height (cm)	161.2 ± 5.6 (146.9 to 177.8)	173.1 ± 7.8 (147.7 to 193.0)
Weight (kg)	62.5 ± 14.2 (42.7 to 115.0)	72.4 ± 15.1 (47.6 to 122.6)
Total fat (kg)	21.0 ± 8.8 (8.0 to 53.6)	15.8 ± 8.8 (3.8 to 45.4)
Total lean (kg)	38.2 ± 6.1 (27.4 to 57.7)	52.7 ± 8.0 (35.8 to 80.2)
BMI (kg/m^2)	24.0 ± 5.1 (16.0 to 41.4)	24.1 ± 4.4 (17.2 to 42.4)
BMI z-score	-0.01 ± 1.1 (-1.7 to 3.6)	0.01 ± 0.9 (-1.4 to 3.9)
CT Vertebral CSA (cm^2)	8.6 ± 1.1 (6.0 to 12.5)	10.9 ± 1.4 (8.2 to 14.7)
CT Vertebral CBD (mg/cm^3)	176 ± 27 (116 to 232)	169 ± 25 (96 to 234)
CT Femoral CSA (cm^2)	5.0 ± 0.7 (3.6 to 7.1)	6.2 ± 0.8 (4.4 to 13.1)
CT Femoral CBA (cm^2)	4.1 ± 0.5 (2.5 to 5.5)	5.0 ± 0.7 (3.3 to 6.7)
DXA Vertebral BMC (g)	12.4 ± 2.1 (8.0 to 21.4)	14.4 ± 3.1 (7.9 to 23.3)
DXA Vertebral BMD (g/cm^2)	1.0 ± 0.1 (0.7 to 1.5)	1.0 ± 0.1 (0.6 to 1.3)
DXA Legs BMC (g)	373 ± 67 (234 to 555)	495 ± 94 (306 to 848)
DXA Legs BMD (g/cm^2)	1.1 ± 0.1 (0.9 to 1.5)	1.3 ± 0.1 (0.9 to 1.8)

Table 2. Correlation coefficients for DXA and CT bone measurements

		CT							
		Females				Males			
		Vertebral		Femoral		Vertebral		Femoral	
		CSA	CBD	CSA	CBA	CSA	CBD	CSA	CBA
DXA	**Vertebral**								
	BMC	.50	.55	.67	.66	.52	.52	.53	.72
	BMD	.28	.72	.56	.59	.34	.72	.47	.67
	Leg								
	BMC	.54	.42	.87	.89	.51	.43	.71	.89
	BMD	.41	.55	.74	.85	.32	.60	.56	.80

All are significant to the P-value < 0.0001

Table 3. Correlation coefficients for DXA bone measurements with lean and fat mass

	Females		Males	
	Lean	Fat	Lean	Fat
Vertebral				
BMC	.60	.35	.63	-.01*
BMD	.54	.46	.58	.13*
Leg				
BMC	.83	.48	.79	.24
BMD	.74	.41	.62	.10*

* Are not significant to the P-value < 0.05

Table 4. Multiple linear regressions showing the simultaneous effects of lean and fat mass after adjusting for leg length/trunkal height on CT bone measurements in the appendicular and axial skeletons of 150 females and 150 males

	Females		Males	
	β	P-value	β	P-value
Vertebral CSA				
Trunk Height	.052	.024	.045	.156
Fat Mass	-.008	.497	-.010	.390
Lean Mass	.111	<.0001	.101	<.0001
Vertebral CBD				
Trunk Height	-.250	.724	-1.384	.052
Fat Mass	.041	.906	-.730	.006
Lean Mass	1.502	.005	1.306	.001
Femoral CSA				
Leg Length	.017	.018	.018	.147
Fat Mass	-.007	.177	.005	.519
Lean Mass	.096	<.0001	.072	<.0001
Femoral CBA				
Leg Length	.000	.966	.000	.128
Fat Mass	-.007	.093	-.010	.034
Lean Mass	.082	<.0001	.066	<.0001

Table 5. Multiple linear regressions showing the simultaneous effects of lean and fat mass after adjusting for leg length/trunkal height on DXA bone measurements in the appendicular and axial skeletons of 150 females and 150 males

	Females		Males	
	β	P-value	β	P-value
Vertebral BMC				
Trunk Height	.157	.001	.134	.029
Fat Mass	-.042	.060	-.146	<.0001
Lean Mass	.211	<.0001	.284	<.0001
Vertebral BMD				
Trunk Height	.002	.361	-.002	.583
Fat Mass	.002	.154	-.003	.005
Lean Mass	.008	<.0001	.011	<.0001
Leg BMC				
Leg Length	2.308	.001	3.653	<.0001
Fat Mass	-.947	.063	-1.527	<.010
Lean Mass	9.706	<.0001	8.803	<.0001
Leg BMD				
Leg Length	-.001	.286	.000	.930
Fat Mass	-.003	.001	-.004	.001
Lean Mass	.017	<.0001	.013	<.0001

Figure 1

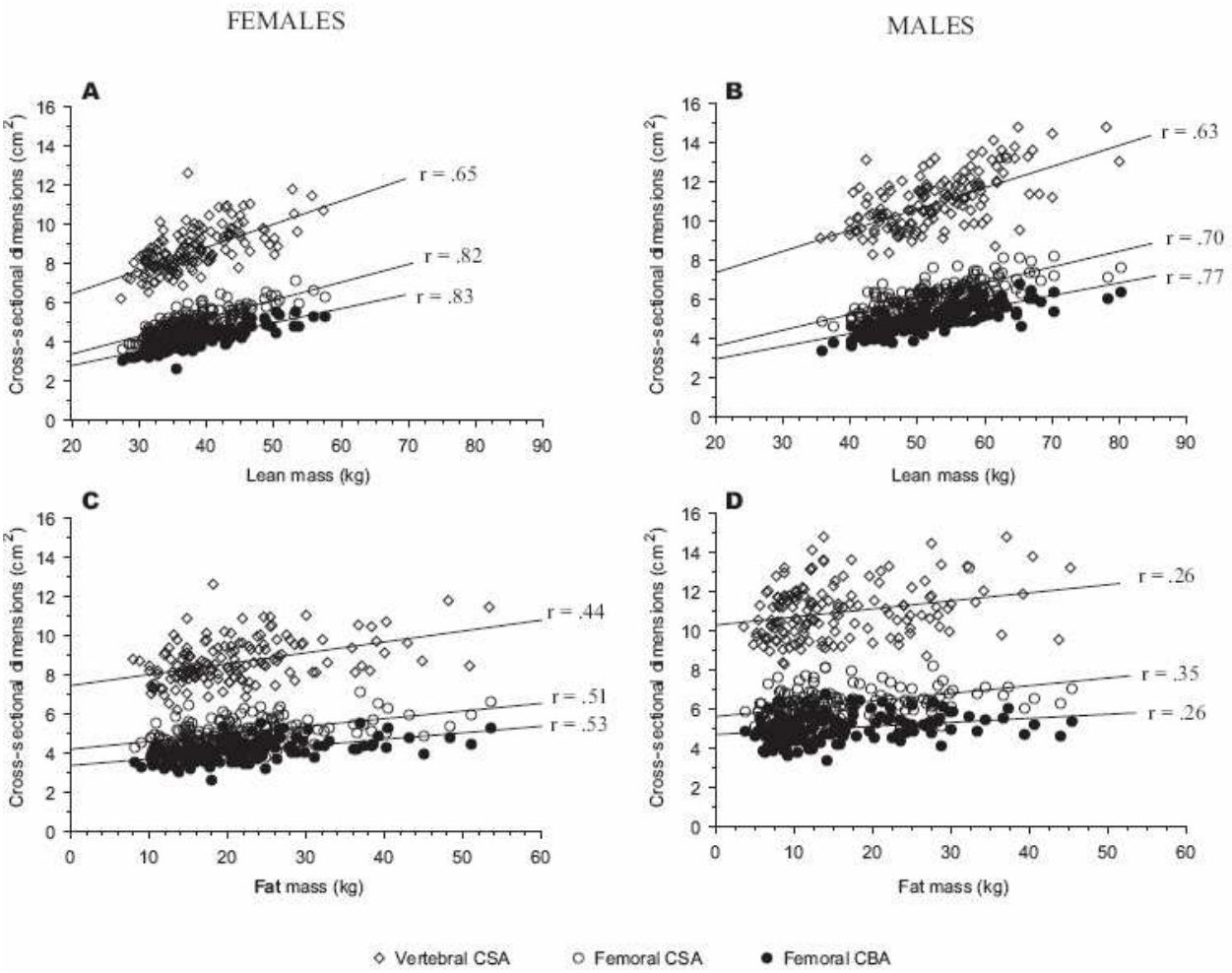
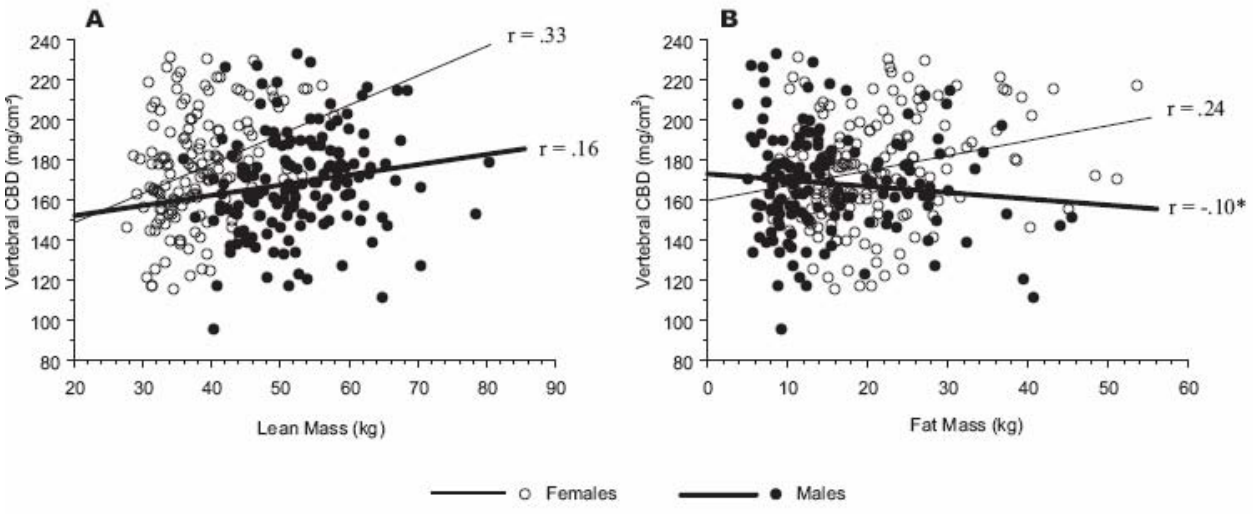

FEMALES
MALES
A
B
C
D
Cross-sectional dimensions (cm²)
Lean mass (kg)
Fat mass (kg)
r = .65
r = .82
r = .83
r = .63
r = .70
r = .77
r = .44
r = .51
r = .53
r = .26
r = .35
r = .26
◇ Vertebral CSA
○ Femoral CSA
● Femoral CBA

Figure 2



* Not significant to the P-value < 0.05